# THE NUTRITIONAL VALUE OF TELANG LIVERS[1]

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TWO FIGURES

(Received for publication January 24, 1945)

Telangiectasis in cattle livers has been noted since 1900 and has been the subject of various reports (Schote, '36, and Carta, '38). The abnormality is characterized by dark red or purplish depressed areas beneath the capsule of the liver which vary in size from a few millimeters to 15 mm. in diameter. On gross sections, the dark areas can be seen distributed through the entire liver, although in some cases only one lobe may be involved. The dark areas are soft and spongy and are usually engorged with blood.

It has been reported by Smith ('40) that nearly 3% of all beef livers inspected each year in the United States are condemned for telangiectasis. In the meat packing industry the common name of these livers is telang livers. Only a few suggestions as to the possible pathogenesis of the condition are available. Experiments at Kansas State College ('40) disclosed a high incidence of telang lesions in the livers from cattle fed high fat rations. Frederick ('43) pointed out that the incidence of telangiectasis tends to be greatest in steers which have been on highly concentrated fattening rations for extended periods of time.

The experiments to be reported in this paper were undertaken with the aim of establishing the nutritional value of these livers and possibly to cast some light on the pathogenesis of the condition. Feeding tests and certain chemical and vitamin analyses were made on representative samples of both normal and telang livers.

## MINERAL AND PROXIMATE COMPOSITION

Twenty-six livers exhibiting severe telang lesions and thirty-three normal livers were selected for the analytical work. The fat content was determined by ethyl ether extraction of dried samples from each liver. The telang livers showed a range of 19.7 to 27.6% while the

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by a grant from the National Live Stock and Meat Board.



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normal livers contained from 15.0 to 21.3% of fat. No significance is attached to these variations in fat content. The iron content of the telang livers ranged from 27.5 to 35.0 mg. and the normal livers from 21.0 to 24.0 mg. per 100 gm. of dry matter. The copper values for the telang livers showed a wider variation from 2.0 to 13.8 mg. per 100 gm. dry matter and were somewhat lower than those for the normal liver 7.0 to 16.0 mg. per 100 gm. The higher iron content of the telang livers may have been due to the blood held in the telangiectatic lesions.

## VITAMIN CONTENT OF LIVERS

*Carotene and vitamin A.* In the same series of twenty-six telang livers, carotene values ranged from 0.2 to 2.15 mg. per 100 gm. of fresh material. The normal livers showed a comparable spread of 0.25 to 2.0 mg. per 100 gm. Of twenty-six telang livers assayed for preformed vitamin A (Davies, '33), eighteen of them had vitamin A values of 10 to 60 mg. per 100 gm. In contrast to this, only one of the thirty-three normal samples had an especially high content, 55 mg., and the majority of the samples contained less than 10 mg. per 100 gm. of fresh liver.

*Ascorbic acid.* Vitamin C determinations by the indophenol technique (Mindlin and Butler, '38) indicated that both normal and telang livers contained about the same concentration, i.e., 15 to 30 mg. per 100 gm. of fresh tissue.

*Choline.* Determination of this substance in the dried livers was made colorimetrically after precipitation of the choline as the reineckate. Values for both types of liver were in the narrow range of 1500 to 1600 mg. per 100 gm. of dried material.

*Pantothenic acid.* The microbiological method for assay showed telang and normal livers to contain from 14 to 18 mg. per 100 gm. on the dry weight basis.

*Niacin.* Microbiological assay for this factor demonstrated that both types of liver contained between 40 and 50 mg. per 100 gm. of dried liver.

*Biotin.* Assays for this compound were also made microbiologically. The one sample of dried telang liver tested had a biotin content of 0.34 mg. while a normal liver assayed at the same time contained 0.63 mg. per 100 gm. It is doubtful whether this variation has any significance.

## FEEDING EXPERIMENTS WITH FRESH, NORMAL AND TELANG LIVER

Preliminary work on the selection of a suitable laboratory animal to study the growth promoting effect and possibly toxicity of telang liver as compared to normal beef liver, indicated that the rat was most de-

sirable. Dogs, cats, chicks and monkeys were also investigated as possible experimental animals.

These early studies revealed that rats could be maintained from weaning to maturity, without any deleterious effects, on rations consisting of fresh normal beef liver supplemented only with 50–100 mg. of calcium carbonate per day. However, it was observed that certain telang livers fed under identical conditions were quite toxic to weanling rats. Animals fed these livers failed to grow, developed severe paralysis of the front and hind quarters which very shortly progressed until the front legs were useless. This paralysis together with bone fractures was observed in 7 to 10 days and the animal usually died within 3 weeks unless the liver feeding was discontinued. These observations suggested that the telang liver contained a specific toxic substance, not present in normal beef liver. Preliminary results (Herbst et al., '44) indicated a relationship between the toxicity and the high vitamin A content. The following experiments were carried out to study this relationship further.

Weanling Sprague-Dawley male albino rats (40–50 gm.) were used to assay forty-eight different telang livers. The livers were obtained from the Oscar Mayer Co. of Madison and from Wilson and Co., Chicago. They were received in a frozen condition and immediately stored at 0°F. Large representative samples of each liver were ground and stored at all times under freezing conditions to prevent spoilage. The degree of telang lesion formation was recorded for each liver. If the lesions comprised about one-half the total liver area, the liver was graded "Very Extensive Lesions"; if the lesions were quite numerous but made up less than one-half of the liver area, the grade "Extensive Lesions" was applied; if the lesions were scattered and limited to only a small part of the total liver area, the liver was graded "Slightly Marked".

The ground liver was placed in the food cups in the frozen condition. The excess was washed out of the cups on the following day and fresh frozen liver was again fed. Fifty to 100 mg. of calcium carbonate were added to the liver fed each rat to correct the undesirable Ca : P ratio (about 1 : 16) existing in beef liver. Two drops of halibut liver oil (diluted 1 : 5 with corn oil) were fed to each rat per week to insure an adequate intake of vitamin D.

Seventeen normal beef livers, obtained from a local butcher shop, were assayed for toxicity under conditions identical to those that existed during the telang liver assay.

The effect of the addition of 25 and 50% of fresh normal liver to toxic telang liver was studied. Equal portions of three normal livers, that had proved non-toxic in previous feeding experiments and of three toxic telang livers were ground together and thoroughly mixed. Mixtures of the same livers containing 25% of normal liver and 75% of toxic telang liver were prepared in a similar manner.

Each of the six mixtures was fed to weanling Sprague-Dawley male rats; three control rats received the undiluted toxic telang liver. Supplements of calcium carbonate and halibut liver oil were fed and in general the conditions that existed during the assay of the livers for toxicity were maintained.

## RESULTS

The results of feeding the forty-eight telang livers, three of which were graded as "Very Extensive", twenty-six as "Extensive" and nineteen as "Slightly Marked", can be summarized as follows: The livers with the extensive or very extensive lesions usually showed toxicity and in a few cases designated as "Slightly Marked" also produced the typical paralysis.

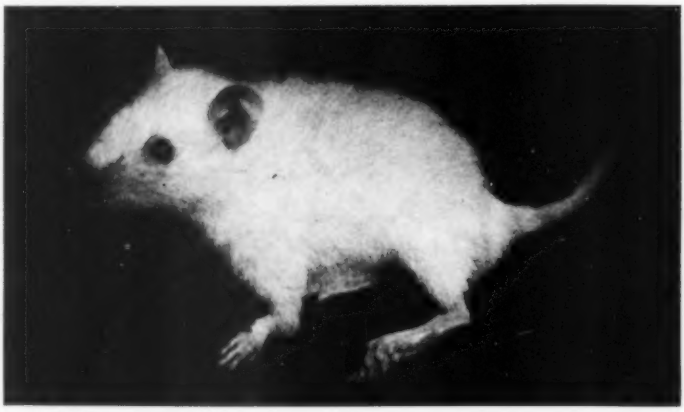

Fig. 1 Weanling rat fed a toxic telang liver plus calcium carbonate ad libitum. The typical paralysis of the legs is evident.

The extreme brittleness of the leg bones appearing in the animals fed telang liver designated as toxic was the most constant symptom observed. Fractures, often multiple, occurred usually in the tibia of the rear legs and in the radii and ulnae of the forelegs. When fractures occurred in the bones of the forelegs, the legs became twisted and the animal would crawl about on the deformed limbs. Figure 1 shows

an animal typical of those which had been on the toxic telang liver diet for 7–10 days.

Growth failure always preceded the bone fragility. If the liver feeding was continued after the onset of the symptoms the animals died, usually within a week. Respiratory involvements were evident in those animals that succumbed to telang liver toxicity. The affected animals would breath laboriously and bloody discharges were often observed around the nostrils. Paralysis of the hind legs was quite common and enuresis occurred frequently.

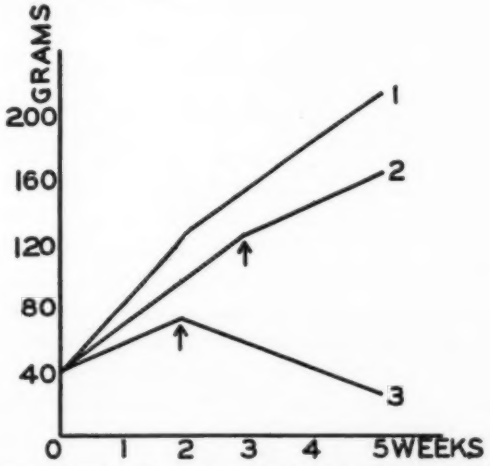


Fig. 2 Growth curves of male albino rats on fresh toxic telang-normal beef liver mixtures.
(1) Received equal portions of normal liver and toxic telang livers.
(2) Received 25% normal liver and 75% toxic telang liver.
(3) Received 100% toxic telang liver.
Arrows indicate time at which bone fractures occurred.

Post mortem examination of the animals revealed severely congested lungs indicating that death may have been due to pneumonia. The livers were unusually yellow but all other organs were normal. Since fatty livers were also observed in animals fed normal liver and nontoxic telang liver, this observation is not of great significance.

None of the seventeen normal livers assayed proved toxic to weanling rats. All animals fed calcium supplemented normal liver as the sole dietary grew extremely well and in no case were symptoms of bone fragility observed.

The results of one experiment in which mixtures of toxic telang liver and normal liver were fed to weanling rats are shown graphically in figure 2. Growth and toxicity data in two additional experiments were

quite similar to the results illustrated. Weanling rats were completely protected from the toxic effects of telang liver if their rations contained 50% of normal beef liver. The inclusion of 25% of normal liver did not afford complete protection. However, the onset of the symptoms of bone fragility was delayed and growth was considerably better than in the case of the animals fed 100% toxic liver.

## VITAMIN A TOXICITY EXPERIMENTS

The literature contains frequent reports of the toxicity of large dosages of vitamin A to laboratory animals and humans. These studies have recently been reviewed by Josephs ('44).

An almost constant feature of hypervitaminosis A in rats is the development of multiple spontaneous fractures of the bones of the extremities. The close similarity of these symptoms to those observed in rats fed toxic telang liver suggested that excessively high stores of vitamin A might be present in certain of the abnormal livers.

The studies referred to above on the comparative vitamin A content which to date have involved over 100 samples of telang and normal beef liver, disclosed that the abnormal livers were consistently higher in vitamin A potency. It was impossible to establish a correlation between animal toxicity and the vitamin A storage in every liver studied since toxicity data were not available for all the samples assayed.

To illustrate the correlation which existed between the toxicity of certain samples of telang liver and their content of vitamin A, several livers of varying vitamin A content were fed to weanling rats. Total vitamin A intakes of the rats were calculated from the vitamin A potency of the liver and the amount ingested per rat per day. Data on six telang livers, two of which were toxic and four non-toxic, are summarized in table 1.

TABLE 1

*Summary of vitamin A content and toxicity of telang livers.*

| DESCRIPTION OF RATION [1] | VITAMIN A CONTENT OF LIVER | INTAKE OF VITAMIN A PER RAT PER DAY [2] | DAYS REQUIRED TO PRODUCE BONE FRACTURES | GROWTH OF RATS |
|---|---|---|---|---|
| | *I.U./100 gm.* | *I.U.* | | *gm./week* |
| Telang liver, no. 43 | 121,000 | 22,990 | 7 | 0 |
| Telang liver, no. 44 | 132,000 | 25,080 | 7 | 0 |
| Telang liver, no. 45 | 68,400 | 12,996 | No fractures | 15 |
| Telang liver, no. 46 | 50,800 | 9,652 | No fractures | 20 |
| Telang liver, no. 47 | 45,200 | 8,588 | No fractures | 29 |
| Telang liver, no. 48 | 59,000 | 11,210 | No fractures | 26 |

[1] Each rat received a daily supplement of 50–100 mg. of calcium carbonate.

[2] Daily intake of vitamin A calculated from food consumption data.

As further demonstration that the toxic symptoms manifested by bone fracturing were related to the high intake of vitamin A, halibut liver oil [2] and also crystalline vitamin A alcohol [3] were fed as supple-

TABLE 2

*The toxicity of crystalline vitamin A and haliver oil supplements to weanling rats.*

| DESCRIPTION OF RATION [1] | TOTAL INTAKE OF VITAMIN A/ RAT/DAY [2] | DAYS REQUIRED TO PRODUCE BONE FRACTURES | | | GROWTH OF RATS IN GM./WEEK | | |
|---|---|---|---|---|---|---|---|
| | I.U. | Rat number | | | Rat number | | |
| | | 1 | 2 | 3 | 1 | 2 | 3 |
| 1. Fresh telang liver, no. 47 plus 5 drops corn oil | 8,588 | | | | 31 | 39 | 13 |
| 2. Fresh telang liver, no. 47 plus 10 drops corn oil | 8,588 | | | | 31 | 33 | 33 |
| 3. Fresh telang liver, no. 47 plus 10,000 I.U. of crystalline vitamin A | 18,588 | 11 | 14 | 11 | 8 | 14 | 2 |
| 4. Fresh telang liver, no. 47 plus 10,000 I.U. of vitamin A as haliver oil | 18,588 | 11 | 11 | 7 | 5 | 3 | 17 |
| 5. Fresh telang liver, no. 47 plus 20,000 I.U. of crystalline vitamin A | 28,588 | 7 | 8 | 3 | 2 | — 4 | 2 |
| 6. Fresh telang liver, no. 47 plus 20,000 I.U. of vitamin A as haliver oil | 28,588 | 7 | 7 | 6 | 1 | — 17 | — 13 |
| 7. Steenbock stock ration plus 20,000 I.U. of crystalline vitamin A | 20,000 | 11 | | 8 | 3 | 23 | 3 |
| 8. Steenbock stock ration plus 20,000 I.U. of vitamin A as haliver oil | 20,000 | | 7 | 12 | 24 | 15 | 0 |

[1] Supplements of corn oil and vitamin A to liver and stock rations are in terms of daily intake per rat.

All animals fed liver rations received daily supplement of 50–100 mg. of calcium carbonate.

[2] Vitamin A supplied by ration plus supplement.

ments to rats in doses reported to be toxic by Josephs (’44). These supplements were fed at levels calculated to furnish 10,000 and 20,000 I.U. of vitamin A in addition to the amount furnished by the basal diet of fresh telang liver. The sample of liver chosen for the basal diet was

[2] Supplied by Abbott Laboratories, North Chicago, Ill. The oil contained 60,000 I.U. of vitamin A per gram.

[3] Obtained from Distillation Products, Inc., Rochester, N. Y.

sample number 48 which previous assay (table 1) had indicated to be non-toxic. The high vitamin A supplements were also fed in conjunction with a natural ration (Steenbock Stock Ration). Three animals in each group were used to establish the toxicity or lack of toxicity of the supplemented ration. The results of this series of experiments are given in table 2.

These data reveal that the toxicity of a liver and its vitamin A content are closely related. Furthermore, the amount of the vitamin ingested by rats which succumbed to the paralysis was in excess of the toxic dosage of 15,000 I.U. per day reported by Josephs ('44).

The results of the experiments with crystalline vitamin A alcohol and halibut liver oil supplements, summarized in table 2, have definitely identified the telang liver toxicity as a hypervitaminosis A.

The toxic effects of vitamin A supplements and of telang liver were identical. Bone fractures were observed in sixteen of the eighteen animals fed vitamin A supplements approximately equivalent to the intake of the vitamin by weanling rats on toxic telang liver rations. The two animals that failed to show symptoms of bone brittleness resisted the feeding of vitamin A supplements and did not always take the entire dose. All the constant symptoms observed in rats fed telang liver (growth failure, respiratory involvements, etc.) were reproduced in the animals receiving toxic doses of vitamin A.

## DISCUSSION

It is clear from the results reported in this paper that the toxic effects resulting from the ingestion of very high amounts of telang liver are due to the accumulation of extra amounts of vitamin A in these livers. At present it is impossible to decide whether the telang liver lesions are the result of the abnormal storage of vitamin A or the increased storage is related to a disturbance in the structure of the liver due to other causes. The high vitamin A content makes the telang liver more valuable from a strictly nutritional point of view since a level high enough to produce hypervitaminosis A would never be used in ordinary rations.

The toxic dosage of vitamin A for growing rats which we have set at approximately 20,000 I.U. per day agrees favorably with the work reported by Rodahl and Moore ('43) and Josephs ('44).

## SUMMARY

Telang and normal livers were quite similar in their content of carotene, ascorbic acid, choline, pantothenic acid, niacin, biotin and of

fat. The iron content of the telang livers was about 50% higher than observed in normal livers. The copper content of the two types of liver showed wider fluctuations but were of the same order.

About 20% of fresh telang livers tested were toxic to weanling rats receiving the calcium supplemented liver as their sole dietary. The symptoms observed in the affected rats were growth failure, paralysis and multiple spontaneous fractures of the bones of the extremities.

The toxicity of certain telang livers was due to their abnormally high stores of vitamin A. When these livers supplemented only with calcium carbonate were fed to rats, the daily intake of the vitamin was in excess of the toxic dosage of 15,000 I.U. per day. Equivalent amounts of crystalline vitamin A alcohol or of vitamin A supplied by halibut liver oil produced identical symptoms of toxicity when fed to weanling rats.

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# BIOMICROSCOPY OF THE EYES IN EVALUATION OF NUTRITIONAL STATUS. CONJUNCTIVAL CHANGES [1]

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(Received for publication February 8, 1945)

During a survey of the nutrition status of a rural population of Wayne County, North Carolina, comprising 120 white families and 80 Negro families, with a total of 1,010 individuals (Milam and Anderson, '44), a study was made to determine whether there was any correlation between the severity and incidence of conjunctival changes detected in these persons by means of a slit lamp, and the level of vitamin A in their diet and in their blood plasma.

That vitamin A deficiency produces conjunctival changes characterized by xerosis, xerophthalmia, and keratomalacia has been recognized for many years. Kruse ('41) has described certain conjunctival changes occurring in 99% of a poor income group. These changes he considers as diagnostic of vitamin A deficiency. He observed clearing of these lesions in nine cases after prolonged therapy with very large doses of vitamin A (100,000 units daily for 6 months or more). Berliner ('42) has criticised Kruse's findings, stating that what Kruse calls Bitot spots are really pingueculae, and differ essentially from true Bitot spots in having the epithelium intact. Certainly Kruse's description more closely fits pingueculae than the usual description of Bitot spots. Kruse reported the highest incidence in older age groups, while true Bitot spots are said to be most common in children. Berliner ('43) could find no conjunctival changes in infants who were on a deficient diet and whose blood vitamin A values were well below normal; and he found such changes in persons with normal blood values. He believes that the changes described by Kruse are essentially degenerative changes associated with age and that pingueculae may be genetically determined.

[1] The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of The Rockefeller Foundation in cooperation with the North Carolina State Board of Health and Duke University School of Medicine.

Pett ('43) gave vitamin A for 3 months to thirty-two patients having conjunctival opacities and observed clearing in four of them. Sixty-three controls showed no change. Either the majority of the cases were not due to vitamin A deficiency or the period of therapy was not sufficiently prolonged.

Kruse divided his conjunctival lesions into three main types: those with gross spots, others with gross signs but no spots, and a third class with signs apparent only by biomicroscope. We have divided our cases into three approximately similar groups, referring to them as severe, moderate, and mild. However, it did not seem that the presence of a discrete spot was always the best criterion of severity; and occasional cases with small, grossly visible spots but with otherwise moderate or mild changes were put in the moderate groups and similarly, cases without discrete spots but with marked changes throughout a large portion of the conjunctiva were included in the severe group. When there was a question as to grouping, we tried to err on the side of conservatism, placing the case in a milder rather than a more severe group.

## RESULTS AND DISCUSSION

Table 1 presents a comparison of blood vitamin A with conjunctival changes. In neither the white nor the colored group is there any indication that low blood vitamin A values are correlated with the presence of conjunctival changes. The lower values in the younger age group for those with mild changes are not significant from a statistical standpoint. Where values for all ages are combined there is a definite tendency for the blood values to be highest in those individuals with conjunctival abnormalities, a tendency which greatly decreases when the data are broken down into age groups. This is probably due largely to the fact that both blood vitamin A values and the severity of conjunctival changes tend to increase with age. It will be seen that the incidence of conjunctival changes shows a regular progression with increasing age to a point where a conjunctiva without changes in an adult is the exception.

Conjunctival changes appear to be more common in the Negroes than in whites, particularly in the younger age groups. In adults the incidence is so high in both races that the total incidence is not greatly different, but the severe grades are definitely more common in Negroes than in whites. This is in spite of the fact that in the families examined the average blood vitamin A values for the two races were not greatly different (whites, 95 I.U.; Negroes, 92 I.U.), and the dietary vitamin A, including carotene (white, 4,850 I.U.; Negroes, 5,540 I.U.), as well

TABLE 1

*Comparison of plasma levels of vitamin A with severity of conjunctival changes.*

| AGE | CATEGORY OF INTEREST | WHITE RACE | | | | | | NEGRO RACE | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Severity of conjunctival changes | | | | Per cent with conjunctival changes | Per cent with severe conjunctival changes | Severity of conjunctival changes | | | | Per cent with conjunctival changes | Per cent with severe conjunctival changes |
| | | None | Mild | Moderate | Severe | | | None | Mild | Moderate | Severe | | |
| *(years)* | | | | | | | | | | | | | |
| 4–9 | Number in group | 60 | 6 | 0 | 0 | 9 | 0 | 58 | 14 | 2 | 0 | 22 | 0 |
| | Mean plasma vitamin A | 73 | 63 | .. | .. | | | 74 | 73 | 83 | .. | | |
| | St. error of the mean | ± 2.9 | ± 5.8 | .. | .. | | | ± 1.8 | ± 4.7 | ± 1.0 | .. | | |
| 10–15 | Number in group | 86 | 26 | 3 | 1 | 26 | 1 | 46 | 40 | 11 | 0 | 53 | 0 |
| | Mean plasma vitamin A | 81 | 78 | 76 | 94 | | | 86 | 87 | 87 | .. | | |
| | St. error of the mean | ± 2.5 | ± 4.2 | ± 2.4 | .. | | | ± 3.3 | ± 2.7 | ± 3.8 | .. | | |
| 15 + Adult male | Number in group | 5 | 19 | 45 | 32 | 95 | 32 | 4 | 16 | 15 | 55 | 96 | 62 |
| | Mean plasma vitamin A | 101 | 105 | 107 | 114 | | | 95 | 94 | 105 | 109 | | |
| | St. error of the mean | ± 13.3 | ± 7.9 | ± 4.3 | ± 4.4 | | | ± 10.3 | ± 5.0 | ± 6.2 | ± 3.7 | | |
| 15 + Adult female | Number in group | 18 | 39 | 48 | 36 | 87 | 26 | 2 | 14 | 28 | 65 | 98 | 59 |
| | Mean plasma vitamin A | 90 | 88 | 100 | 98 | | | 82 | 97 | 84 | 92 | | |
| | St. error of the mean | ± 4.0 | ± 3.6 | ± 3.7 | ± 4.2 | | | ± 8.8 | ± 6.7 | ± 4.2 | ± 3.1 | | |
| All ages | Number in group | 169 | 90 | 96 | 69 | 60 | 16 | 110 | 84 | 56 | 120 | 70 | 32 |
| | Mean plasma vitamin A | 80 | 87 | 103 | 105 | | | 80 | 88 | 90 | 100 | | |
| | St. error of the mean | ± 1.8 | ± 2.9 | ± 2.8 | ± 3.2 | | | ± 1.8 | ± 2.3 | ± 3.0 | ± 2.5 | | |
| Per cent persons with plasma vitamin A below 70 I.U. | Number in group | 60 | 6 | 0 | 0 | | | 58 | 14 | 2 | 0 | | |
| | Age 4–9 yrs. | 50% | 67% | .. | .. | | | 46% | 57% | 0% | .. | | |
| | Number in group | 86 | 26 | 3 | 0 | | | 46 | 40 | 11 | 0 | | |
| | Age 10–15 yrs. | 30% | 38% | .. | .. | | | 20% | 18% | 0% | .. | | |
| | Number in group | 23 | 58 | 93 | 68 | | | 6 | 30 | 43 | 120 | | |
| | Adults 15 yrs. + | 17% | 16% | 11% | 6% | | | 0% | 10% | 21% | 13% | | |
| | Number in group | 169 | 90 | 96 | 68 | | | 110 | 84 | 56 | 120 | | |
| | All ages | 36% | 26% | 10% | 6% | | | 33% | 21% | 16% | 13% | | |

TABLE 2

*Comparison of mean vitamin A dietary intake with severity of conjunctival changes.*

| AGE | CATEGORY OF INTEREST | WHITE RACE | | | | NEGRO RACE | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Severity of conjunctival changes | | | | Severity of conjunctival changes | | | |
| | | None | Mild | Moderate | Severe | None | Mild | Moderate | Severe |
| *(years)* | | | | | | | | | |
| 4–9 | Number in group | 56 | 6 | 0 | 0 | 56 | 10 | 2 | 0 |
| | Mean vitamin A intake in international units | 3550 | 3268 | .. | .. | 5709 | 3082 | 4915 | .. |
| | St. error of the mean | ± 232 | ± 842 | .. | .. | ± 690 | ± 666 | ± 1603 | .. |
| 10–15 | Number in group | 75 | 19 | 4 | 1 | 44 | 41 | 10 | 0 |
| | Mean vitamin A intake in international units | 4212 | 4856 | 9910 | 2334 | 4726 | 5945 | 8738 | |
| | St. error of the mean | ± 399 | ± 651 | ± 3220 | .. | 473 | 680 | 1032 | .. |
| 15 + Adult male | Number in group | 5 | 19 | 39 | 28 | 4 | 14 | 14 | 51 |
| | Mean vitamin A intake in international units | 2740 | 5074 | 6115 | 5340 | 6851 | 5651 | 6124 | 5835 |
| | St. error of the mean | ± 953 | ± 674 | ± 624 | ± 613 | ± 2190 | ± 1267 | ± 902 | ± 683 |
| 15 + Adult female | Number in group | 18 | 36 | 47 | 31 | 3 | 13 | 28 | 56 |
| | Mean vitamin A intake in international units | 4726 | 4763 | 4560 | 5284 | 3887 | 6339 | 4177 | 5682 |
| | St. error of the mean | ± 669 | ± 525 | ± 368 | ± 770 | ± 882 | ± 971 | ± 477 | ± 753 |
| All ages | Number in group | 154 | 80 | 90 | 60 | 107 | 78 | 54 | 107 |
| | Mean vitamin A intake in international units | 3984 | 4747 | 5471 | 5261 | 5296 | 5591 | 5554 | 5755 |
| | St. error of the mean | ± 231 | ± 333 | ± 383 | ± 493 | ± 425 | ± 476 | ± 459 | ± 513 |
| Per cent of persons with intakes below 2500 I.U. of vitamin A daily | | 27 | 28 | 17 | 25 | 24 | 23 | 22 | 29 |

as blood carotene (white, 286 I.U.; Negro, 422 I.U.) was definitely higher in the Negroes.

Table 2 presents a comparison of average daily vitamin A intakes with the conjunctival changes. Again there is no evidence of correlation with the incidence or severity of the conjunctival changes. Since the average vitamin A intake approaches the daily allowance recommended by the National Research Council, one would not expect an incidence of lesions of vitamin A deficiency as high as the incidence of conjunctival changes encountered in this study.

We realize that the exact interpretation of blood vitamin A values is still doubtful and that the blood values are by no means invariably correlated with the presence or severity of the clinical lesion. Probably even more than with dietary intakes, however, it would seem logical to expect some correlation between blood values and the signs of avitaminosis in a rather large group of persons. That correlation of both blood and dietary vitamin A with the conjunctival lesion is absent impairs seriously the validity of the view that a conjunctival lesion is evidence of vitamin A deficiency.

The inaccuracy, lack of sensitivity, and cumbersomeness of most of the present methods of evaluating nutritional status is fully recognized. We therefore feel some disappointment that slit lamp examination of the eyes has not thus far, in our experience, served as useful a purpose as was at first hoped.

## SUMMARY

In a nutrition survey of a population group numbering 1,010, no correlation was observed between the severity and incidence of conjunctival changes detected with the slit lamp, and the level of vitamin A in the diet or in the blood plasma.

The frequency and severity of conjunctival changes were greater in the Negro than in the white.

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# BIOMICROSCOPY OF THE EYES IN EVALUATION OF NUTRITIONAL STATUS. CORNEAL VASCULARIZATION [1]

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(Received for publication February 8, 1945)

There is good experimental and clinical evidence that certain vitamin deficiencies produce pathological changes in the superficial structures of the eye. Vascularization of the cornea in riboflavin deficiency in rats was demonstrated by Bessey and Wolbach ('39) and in humans by Sydenstricker et al. ('40).

Although few students of the problem seriously doubt the production of corneal invasion by riboflavin deficiency, the specificity of this sign has been seriously questioned by recent work. Sandstead ('42) was unable to produce regression of corneal vascularization with large doses of riboflavin (15 mg. daily for 60 days). Scarborough ('42) observed no improvement under riboflavin therapy except in persons with other evidence of deficiency disease. Youmans et al. ('42) found no correlation of corneal vascularization with dietary intake of riboflavin or with other probable signs of riboflavin deficiency. Machella and McDonald ('43) saw some temporary improvement under riboflavin therapy, but all their patients relapsed even while riboflavin was being taken. Pett ('43) observed a high percentage of clearing under riboflavin therapy (57%) but noted also a large percentage of clearing in the control group (42%). Tisdall et al. ('43) reported definite improvement in the majority of patients with severe degrees of invasion. Their study was made with aviators subjected to rather unusual light conditions; the severe cases which they treated were possibly more likely to have been caused by riboflavin deficiency than the milder degrees more commonly seen.

Subsistence of humans on diets presumably deficient with regard to riboflavin has not been observed to result in corneal vascularization.

[1] The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of The Rockefeller Foundation in cooperation with the North Carolina State Board of Health and Duke University School of Medicine.

Sebrell and Butler ('38, '39) in their production of clinical riboflavin deficiency in man did not observe corneal changes. Boehrer et al. ('43) were unable to find corneal vascularization in six persons who received only 0.47 mg. of riboflavin daily in their diet for a period of 5 weeks, although one of the control subjects developed this condition, which persisted for 5 weeks in spite of a normal diet and 9 mg. of riboflavin daily. Williams et al. ('43) failed to observe vascularization or other evidence of riboflavin deficiency in four patients who had subsisted on a diet containing 0.21 mg. riboflavin per 1,000 cal. for 288 days.

Vail and Ascher ('43) emphasize the presence of a normal pericorneal plexus of vascular arcades and believe that much of the so-called corneal vascularization is only engorgement of this normal pericorneal plexus. They prefer the term "concentric collateral vessels." They observed a higher incidence of this condition in patients taking a good diet than in those taking a poor one. They observed true corneal invasion only in patients with evidence of previous corneal disease. They feel that to regard "concentric collaterals" as a specific sign of riboflavin deficiency does not seem justified, although it might be a factor in some cases.

Corneal vascularization has been observed in several other deficiency states, namely vitamin A deficiency (Bessey and Wolback, '39), tryptophan deficiency (Trotter and Day, '42; Albanese and Buschke, '42; and Albanese et al., '43), lysine deficiency (Trotter and Day, '42), zinc deficiency (Follis et al., '41), sodium deficiency (Follis et al., '42), and deficiency of an unidentified component of the vitamin B complex (Gyorgy et al., '42). Corneal vascularization has also been reported to be produced by administration of thallium (Ginsberg and Buschke, '23) and of large doses of nicotinic acid (Gregory, '43). The question of the validity of corneal invasion as a measure of riboflavin deficiency has been the subject of several recent reviews (Nutrition Reviews, '43, '44).

Because of the accessibility of the superficial eye structures, their examination by means of the slit lamp or biomicroscope has been recommended, particularly by Kruse ('42), as a convenient and accurate method of detecting mild degrees of deficiency of riboflavin and vitamin A.

In connection with a survey of the nutritional status of the population of the rural section of Wayne County, North Carolina, which was carried out between July 1942 and June 1943, and included 120 white families and 80 Negro families (a total of 1,010 individuals), we made slit lamp examinations of the eyes of all persons who were capable of

cooperating in such a test. This paper discusses the incidence of corneal vascularization in these persons, as compared with their riboflavin intake. A report of the general aspects of the survey has been published elsewhere (Milam and Anderson, '44). The families included in the survey were selected on the basis of statistical principles to represent correct proportions from geographical areas and from racial and economic groups.

## METHODS

The persons given the slit lamp test were, as a rule, over 4 years old, since satisfactory examinations of this kind were usually not possible in children under that age. From a 7-day food intake record on each person, the daily average intake of the various nutrients was calculated. Corneal invasion was classified as follows:

1. Increased vascularity, and engorgement of the limbic plexus, with some capillaries just appearing to cross the sclero-corneal junction. We have found it difficult to be certain always of the exact location of this junction, capillaries at times appearing to be in the cornea when the slit light was directed at one angle and in the very thin portion of sclera at the margin of the cornea when the light was at a different angle. Vail and Ascher ('43) have emphasized the fact that the final vessels may appear to be in the corneal tissues when they are really only in the transparent tissue that forms the conjunctivo-scleral wedge. There have been included in this group 1, however, only those cases where the capillaries appeared quite definitely to cross the scleral margin.

2. Invasion of the cornea by capillaries to a considerable distance, but with penetration less than a fourth of the distance from the edge of the cornea to its center.

3. Invasion of the cornea by capillaries to a distance between a fourth and a half that to the center of the cornea.

4. Invasion of the cornea by vessels half or more of the distance to its center. Only one case with this degree of severity was seen in the survey.

The degree of penetration of the blood vessels is not the only measure of the severity of corneal vascularization. Certainly the extent of the periphery of the cornea involved and the amount of proliferation and engorgement of vessels is also of importance. For purposes of classification, however, the degree of penetration seems to be the most reliable and useful criterion.

## RESULTS AND DISCUSSION

Data concerning the incidence of corneal invasion for various age groups of the white and colored persons, as compared with their mean riboflavin intake were obtained (table 1). It will be noted that there is no correlation between the riboflavin intake and the incidence or severity of the corneal invasion. The one white adult female with grade 3 invasion did have a low riboflavin intake; but no great significance can be attached to this one case, particularly when persons with even lower intakes were found in all the other groups. The incidence of invasion in the white persons is high in all age groups, the least incidence being in adult males and the highest in the 10- to 15-year age group, although the significance of these differences may be questioned.

The most striking feature apparent in a comparison of the two classes is the markedly lower incidence of corneal invasion in the Negro. As among the whites, the highest incidence in Negroes is in the 10- to 15-year age group and the lowest is in adult males. We have no adequate explanation for this difference in incidence between Negroes and whites. Youmans et al. ('42) also observed the markedly lower incidence in the Negro and suggest that this relative freedom from corneal invasion may be related to their pigmentation. One factor in the adult group may be the higher incidence of arcus senilis in the Negro. In our survey this lesion appeared about eight times as frequently in the Negro as in the white. Sydenstricker et al. ('40) have commented on the absence of corneal invasion in persons with an arcus, and we have had the same experience. However, the incidence of an arcus in our study was not sufficient to have any great effect on the incidence of vascularization. One wonders whether the marked difference observed by Vail and Ascher ('43) in the incidence of vascularization in Cincinnati (45%), as compared with that in Birmingham (13%), may not have been partially due to the greater Negro population in the latter region.

There is a greater difference in the mean riboflavin intakes for the various groups of Negroes than for the groups of whites, due partially at least to the small number of cases in some of the positive groups. However, none of these differences could be shown to be statistically significant.

Although the number is too small to permit conclusions, it may be significant that the three Negroes with grades 3–4 invasion had low intakes of riboflavin. We feel that in the absence of definitive eye disease, the severer grades of invasion are much more likely to be caused by

TABLE 1

*Comparison of riboflavin dietary intake (in μg.) with incidence of varying degrees of corneal vascularization.*

| AGE | CATEGORY OF INTEREST | WHITE RACE | | | | | | NEGRO RACE | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Extent of corneal invasion | | | | Per cent with invasion | Per cent with grade II and III invasion | Extent of corneal invasion | | | | | Per cent with invasion | Per cent with grade II and III invasion |
| | | None | I | II | III | | | None | I | II | III | IV | | |
| *(years)* | | | | | | | | | | | | | | |
| 4–9 | Number in group | 24 | 29 | 9 | 0 | 61 | 15 | 55 | 10 | 2 | 0 | 0 | 18 | 3 |
| | Mean riboflavin intake in micrograms | 924 | 983 | 991 | .. | | | 825 | 921 | 689 | .. | .. | | |
| | St. error of the mean | ± 75 | ± 24 | ± 122 | | | | ± 52 | ± 101 | ± 230 | .. | .. | | |
| 10–15 | Number in group | 24 | 47 | 21 | 9 | 76 | 30 | 67 | 22 | 3 | 1 | 0 | 28 | 4 |
| | Mean riboflavin intake in micrograms | 1144 | 1160 | 1192 | 984 | | | 1052 | 1003 | 1242 | 508 | .. | | |
| | St. error of the mean | ± 115 | ± 88 | ± 120 | ± 147 | | | ± 58 | ± 149 | ± 328 | .. | .. | | |
| 15 + Adult male | Number in group | 38 | 43 | 12 | 0 | 59 | 13 | 77 | 3 | 2 | 1 | .. | 7 | 4 |
| | Mean riboflavin intake in micrograms | 1280 | 1314 | 1050 | .. | | | 984 | 1731 | 1192 | 624 | .. | | |
| | St. error of the mean | ± 89 | ± 116 | ± 159 | .. | | | ± 58 | ± 297 | ± 130 | .. | .. | | |
| 15 + Adult female | Number in group | 50 | 55 | 23 | 1 | 61 | 19 | 82 | 14 | 5 | 0 | 1 | 20 | 6 |
| | Mean riboflavin intake in micrograms | 945 | 1047 | 937 | 703 | | | 845 | 1090 | 830 | .. | 665 | | |
| | St. error of the mean | ± 60 | ± 69 | ± 94 | | | | ± 49 | ± 150 | ± 124 | | .. | | |
| All ages | Number in group | 136 | 174 | 65 | 10 | 65 | 20 | 281 | 49 | 12 | 2 | 1 | 19 | 4 |
| | Mean riboflavin intake in micrograms | 1070 | 1133 | 1048 | 956 | | | 929 | 1056 | 970 | 566 | 665 | | |
| | St. error of the mean | ± 43 | ± 44 | ± 62 | ± 138 | | | ± 28 | ± 88 | ± 126 | .. | .. | | |
| Per cent of persons with riboflavin intakes below 1 mg daily | | 52 | 50 | 55 | 60 | | | 62 | 61 | 58 | 100 | 100 | | |

riboflavin deficiency than the milder types so commonly seen. However, many persons with still lower intakes had no invasion.

The one person with grade 4 invasion also had an angular cheilosis suggestive of riboflavin deficiency. Twelve other persons seen in this survey had angular cheilosis suggestive of riboflavin deficiency. Of these, 50% had no vascularization of the cornea and the rest had grade 1 only. We do not consider that cheilosis is a pathognomonic sign of riboflavin deficiency, but we believe that it is more likely to indicate such deficiency than is corneal invasion. Unfortunately, conditions in this survey were not favorable to a therapeutic test, but in other work we have seen persons without corneal invasion who had rather severe angular cheilosis which responded to riboflavin therapy. We have also seen persons with both cheilosis and corneal invasion in which the cheilosis responded to riboflavin therapy and the invasion did not.

There were nineteen white and nine colored persons who showed tongue changes suggestive of vitamin B complex deficiency. Of the nineteen whites, seven had grade 1, four had grade 2, and one had grade 3 corneal invasion; the rest had none. Eight of the nine colored persons were examined by slit lamp. Six showed no invasion, one showed grade 1 invasion, and the other had grade 2 invasion.

We do not doubt that riboflavin deficiency can produce corneal invasion and that in some instances, along with other evidence of riboflavin deficiency, this sign may furnish confirmatory evidence of the deficiency. However, it appears doubtful whether any very large percentage of cases, particularly those of milder degree so commonly seen, are due to the lack of riboflavin. We realize that there are many unavoidable errors encountered in the taking and calculating of diet records and that even if the diet record is entirely accurate, the recent dietary intake does not always reflect the previous intake responsible for the avitaminosis. Nevertheless, in a large group of individuals, some correlation between the presence of an avitaminotic lesion and the dietary intake is to be expected. Its complete absence in the persons included in our study casts doubt on the value of corneal invasion as a measure of riboflavin deficiency in population groups. The additional fact that there was no correlation between corneal invasion and other physical signs usually considered as suggestive of riboflavin deficiency, also leads us to doubt the diagnostic value of corneal invasion as the sole criterion of riboflavin deficiency. The development of corneal vascularization on diets low but not exceedingly low in riboflavin, as in this study, may well depend on the presence or absence of other

conditioning factors which were not recorded in this study. The search for such factors would be an enterprise well worth while.

## SUMMARY

In a nutrition survey of a rather large population group, no correlation could be found between corneal invasion and the level of riboflavin in the diet. There was also no correlation with other signs suggestive of riboflavin deficiency.

Corneal invasion was found to be much more frequent in the white race than in the Negro race.

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# THIAMINE, RIBOFLAVIN AND NICOTINIC ACID RETENTION IN PREPARATION OF OVERSEAS HAMS AND BACONS[1]

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(Received for publication March 23, 1945)

The present report is based on a study of retention of thiamine, riboflavin and nicotinic acid in the preparation of overseas smoked hams and bacons. These hams and bacons were more heavily salted than the ordinary commercial products to prevent deterioration in shipping to the armed forces. The study was undertaken to study vitamin retention after soaking and cooking of these cured meats according to the directions issued by the Office of the Quartermaster General. A study of vitamin losses in cooking of regular commercially cured hams has been reported by Schweigert, McIntire and Elvehjem ('43).

## EXPERIMENTAL

Five overseas hams, weighing 13–14 lbs. each, were prepared according to the army directions and samples taken for analysis at each step. Care was taken to obtain each time at least two large representative samples from each ham. Three of the hams were prepared as whole hams, two as sliced hams. The directions for soaking and cooking were as follows:

*Whole ham.* Scrub, scrape or brush to remove mold. Trim as necessary (weight). Cover ham with warm water and let soak over night. Drain. Rinse salty sediment from kettle. Cover ham with water. Bring to boiling point, then simmer for at least 3 hours, changing the water at end of each hour. Rinse salty sediment from bottom of kettle each time. During final hour of cooking, add 1 pound of sugar to water in which ham is simmering. Remove ham from water. Skin. Serve boiled, baked, sliced and fried. Baked ham: Place ham in pan, fat side up.

[1] This study was conducted with the cooperation of and published with the approval of the Office of the Quartermaster General, and was supported by the Committee on Food Composition of the National Research Council and the Nutrition Foundation, Inc.

Published with the approval of the Monographs Publication Committee, Oregon State College. Research paper no. 92, School of Science, Department of Chemistry.

Roast uncovered at 300°F. (slow oven) allowing 18 to 25 minutes to the pound. Fried ham: Place slices of ham in hot frying pan and cook in their own fat until brown on both sides.

*Sliced ham.* Scrub, scrape or brush to remove mold Slice into ½-inch slices. Trim, removing rind and excess fat. Soak slices of ham in warm water for 4 hours, changing the water every hour and rinsing salty sediment from bottom of kettle each time. Remove ham slices from water. Drain well. Bake or fry slices of ham.

The hams were weighed after each process to correct for changes in weight. Vitamin retentions are based upon original and final weights of the ham, with appropriate correction for the weight of the bones. The drippings from baked ham were retained for analysis. The whole hams were baked 18, 21, and 25 minutes per pound, respectively, to show possible differences due to cooking time. These are reflected in the extreme range of vitamin content of the drippings.

*Bacon.* Six bacon slabs, weighing 14–16 lbs. each, were prepared according to the following directions: Cut bacon into slices (7 or 8 per inch) and soak in solution of sugar for 2 hours before frying. Use 1 gallon of water containing 1.5 lbs. of sugar for each 18 lbs. of bacon. When frying, care should be taken not to overcook.

Samples of bacon were analyzed as the original cuts, after soaking, and after soaking and frying. Representative samples (7–12 slices) were obtained by combining each third slice of serial cuts for each of the above analyses. Two sections of two bacons were analyzed separately, and two sets of pooled slices from the remaining four bacons taken for assay. The bacon samples were weighed after each process, as was done with the hams. The changes in weight were taken into account in calculating vitamin retention.

## METHODS

All samples, except drippings, were ground four times in a meat grinder prior to analysis. Thiamine assays were made on enzymatically digested samples, using Lactobacillus fermenti (Sarett and Cheldelin, '44). Samples for riboflavin analysis were autoclaved with 0.1N HCl for 15 minutes (Strong and Carpenter, '42) and assayed by the method of Snell and Strong ('39), using the glucose and acetate concentrations suggested by Stokes and Martin ('43). Samples were digested for nicotinic acid analysis by autoclaving for 30 minutes with 1N $H_2SO_4$ and, after neutralization and filtration, assayed using Lactobacillus arabinosus 17–5 by the method of Sarett, Pederson and Cheldelin ('45). The extraction procedures and the assay methods used have been thoroughly

checked and have been found to give valid results as compared to other available techniques.

## RESULTS

In table 1 the results on the hams and bacons are summarized. The vitamin content at each stage of preparation is given and the per cent retention calculated on the basis of the original and final weights of the sample. Although only two sliced hams were used in the study, ranges and average figures are given for these, since two samples were taken from each ham.

The overnight soaking of whole hams removed very little thiamine, riboflavin or nicotinic acid. However, the subsequent boiling reduced the average retention of these three vitamins to 72, 79, and 79% respectively. Slices of boiled ham which were then fried, lost another 10–15% of the original content of each of the three vitamins, whereas baking of the whole ham destroyed about 20% of the thiamine but had very little effect on the other vitamins. The drippings contained only a few per cent of any of the vitamins. The wide variation in vitamin content of the drippings may have been due in part to the difference in baking time for each of the three hams, i.e., 18, 21, and 25 minutes per pound. The baked ham, exclusive of drippings, retained 50, 79, and 71% of the original thiamine, riboflavin and nicotinic acid, respectively.

The greatest loss of vitamins with sliced ham was in the preliminary soaking, where reductions in thiamine, riboflavin and nicotinic acid content averaged 41, 15, and 42%, respectively. Baking of these soaked slices caused a little more destruction of riboflavin than frying, while the losses of thiamine and nicotinic acid were about the same for both cooking methods. The thiamine, riboflavin and nicotinic acid retention in baked slices of ham were 47, 68, and 47%, respectively.

Schweigert, McIntire and Elvehjem ('43) studied vitamin losses in cooking commercially cured hams. They found about the same concentration of nicotinic acid in cured hams as is reported here, but less thiamine and riboflavin. They report retentions after roasting of 58, 74 and 79% for thiamine, riboflavin, and nicotinic acid, respectively. These figures agree with the vitamin content reported here for baked ham, 50, 79, and 71%. However, Schweigert et al. ('43) also found 10–15% of the vitamins in the drippings in contrast to the small amounts observed in the present paper. The high vitamin content of those drippings appears to correspond to the losses we observed in the preliminary soaking and boiling of the overseas hams and may represent a portion of the vitamin which is less firmly bound than that which is retained in the cooked meat.

TABLE 1

*Thiamine, riboflavin and nicotinic acid retention in soaking and cooking of overseas hams and bacons.*

| SAMPLE | WEIGHT RETENTION, PER CENT | | VITAMIN CONTENT IN μG. PER GM. | | | | | | VITAMIN RETENTION IN PER CENT | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Thiamine | | Riboflavin | | Nicotinic acid | | Thiamine | | Riboflavin | | Nicotinic acid | |
| | Range | Av. | Range | Av. | Range | Av. | Range | Av. | Range | Av. | Range | Av. | Range | Av. |
| *Whole ham (3)* | | | | | | | | | | | | | | |
| As received | | | 8.5–10.6 | 10.1 | 2.0–2.9 | 2.5 | 20.0–40.8 | 31.1 | | | | | | |
| Soaked | 100–102 | 101 | 8.1–11.6 | 9.5 | 1.9–2.6 | 2.2 | 18.6–40.0 | 30.5 | 81–100 | 94 | 90–95 | 93 | 94–99 | 97 |
| Boiled | 82–92 | 86 | 7.3– 9.7 | 8.5 | 1.9–2.2 | 2.1 | 16.7–34.6 | 28.8 | 59–83 | 72 | 66–95 | 79 | 69–93 | 79 |
| Slices, fried | 55–71 | 64 | 7.6– 9.0 | 8.3 | 2.3–2.7 | 2.5 | 27.3–35.4 | 32.5 | 49–58 | 55 | 55–75 | 67 | 61–75 | 68 |
| Baked | 53–63 | 61 | 7.1–10.1 | 8.2 | 2.7–3.2 | 3.0 | 28 –44.2 | 35.3 | 43–58 | 50 | 69–85 | 79 | 66–74 | 71 |
| Drippings, baked | 6–11 | 8 | 0 – 5.0 | 3.0 | 0.6–1.1 | 0.8 | 4.8–48.4 | 25.0 | 0–4 | 2 | 2–4 | 3 | 2–8 | 6 |
| *Sliced ham (2)* | | | | | | | | | | | | | | |
| As received | | | 9.5–11.6 | 10.8 | 2.3–2.5 | 2.4 | 24.3–27.2 | 25.5 | | | | | | |
| Soaked | 100–105 | 102 | 6.0– 6.3 | 6.2 | 1.9–2.1 | 2.0 | 13.6–14.9 | 14.4 | 54–69 | 59 | 76–91 | 85 | 55–61 | 58 |
| Fried | 55–75 | 65 | 7.0– 8.6 | 7.7 | 2.7–3.3 | 2.9 | 18.5–22.1 | 20.4 | 40–57 | 47 | 75–80 | 78 | 45–61 | 52 |
| Baked | 59–70 | 65 | 7.1– 9.0 | 7.8 | 2.3–2.9 | 2.5 | 16.2–19.7 | 18.4 | 42–56 | 47 | 64–71 | 68 | 46–50 | 47 |
| Drippings, baked | 19, 10 | | 1.3, 6.7 | | 0, 1.6 | | 12.7, 11.7 | | 2, 7 | | 0, 7 | | 10, 5 | |
| *Bacons (6)* | | | | | | | | | | | | | | |
| As received | | | 2.7– 4.1 | 3.3 | 0.9–1.2 | 1.0 | 11.5–14.5 | 12.6 | | | | | | |
| Soaked | 104–106 | 105 | 1.3– 2.2 | 1.7 | 0.6–1.1 | 0.75 | 4.2– 7.9 | 5.5 | 47–77 | 52 | 69–100 | 78 | 36–69 | 42 |
| Fried | 22–31 | 25 | 1.7– 5.5 | 2.7 | 2.1–3.9 | 2.85 | 10.4–19.6 | 14.7 | 15–41 | 20 | 61–100 | 72 | 21–40 | 30 |
| Drippings, fried | 56–57 | 56 | 0, 0 | | 0, 0 | | 1.7, 0.9 | | 0, 0 | | 0, 0 | | 8, 4 | |

After frying sliced ham, Schweigert et al. ('43) found a retention of 86, 77, and 85%, respectively, of thiamine, riboflavin, and nicotinic acid, and 7–10% more in the drippings. Since the slices we used for frying had already lost part of their vitamin content in previous soaking, or soaking and boiling, it is difficult to compare the present results with their figures. However, it can be seen from the data of table 1 that although the final vitamin retentions for fried ham were low, the actual percentages lost in the frying alone are only from 12–17% for thiamine, 0–8% for riboflavin, and 6–11% for nicotinic acid.

The soaking of sliced bacon removed 48, 22, and 58% of the original thiamine, riboflavin, and nicotinic acid content, respectively. Frying had little further effect on the riboflavin content but destroyed a little more nicotinic acid and most of the thiamine. Only 20% of the original thiamine remained which is comparable to the 13% retention reported for one sample of ordinary bacon by Lane, Johnson, and Williams ('42). However, the 72 and 30% remaining of the riboflavin and nicotinic acid respectively are quite lower than the values of about 100% retention for these vitamins reported by Cheldelin, Woods, and Williams ('43) on unsoaked samples. The major portion of the loss of these two vitamins shown here was in the preliminary soaking. A small part (only a few per cent) of the nicotinic acid was present in the drippings, but no thiamine or riboflavin was found.

## SUMMARY

The thiamine, riboflavin and nicotinic acid contents of heavily salted overseas hams and bacons were studied at different stages of cooking.

Average retentions of thiamine, riboflavin, and nicotinic acid after soaking and boiling of whole hams were 72, 79, and 79%, respectively. Subsequent frying reduced the remaining vitamins to 55, 67, and 68%, respectively, whereas after baking the comparable figures were 50, 79, and 71%. Only a small part (few per cent) of any of the vitamins were found in drippings of the baked ham.

Soaking of sliced ham left only 59, 85, and 58%, respectively, of the original thiamine, riboflavin, and nicotinic acid contents. Retentions after frying these slices were 47, 78, and 52%, while after baking 47, 68, and 47%, respectively, of the thiamine, riboflavin, and nicotinic acid were found.

The losses of these vitamins on soaking of overseas bacon were large, leaving only 52, 78, and 42% of the original thiamine, riboflavin, and nicotinic acid. The amounts of these vitamins remaining after frying were 20, 72, and 30%, respectively.

ACKNOWLEDGMENT

The authors wish to thank Margaret J. Bennett and Thomas R. Riggs for their technical assistance.

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# THE RIBOFLAVIN, NIACIN AND THIAMINE CONTENT OF DRIED LEGUMINOUS SEEDS [1]

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(Received for publication February 12, 1945)

Recent world conditions have given a considerable impetus to the use of legumes as a source of dietary protein. Because of this their vitamin content has become of interest. A review of the literature disclosed certain definite gaps in our knowledge of the B-vitamin content of legumes. With the exception of soybeans, blackeye peas and peanuts very few data are to be found; especially is this true of riboflavin values, and to a lesser extent of niacin values. Moreover, some of the results recorded may be considered questionable, since the analyses were performed before the introduction of the more accurate chemical and microbiological procedures. In view of this a study has been made to determine the riboflavin, niacin and thiamine content of dried leguminous seeds using recent and improved methods.

Some of the more important papers on the vitamin content of legumes appearing in the literature are presented by Fixsen and Roscoe ('38), Booher and Hartzler ('39), Munsell ('40, '42, '43), Halverson and Sherwood ('40), Kelly, Dietrich and Porter ('40), Higgins, Holley, Pickett and Wheeler ('41), Sherman ('41), Booher, Hartzler and Hewston ('42), Teply, Strong and Elvehjem ('42), Burkholder ('43), Russell, Taylor and Beuk ('43).

## EXPERIMENTAL

Samples of dried, unprocessed legumes were obtained from Agronomy Departments of many universities throughout the United States, from seed dealers, and on the open market. All samples were ground in a Wiley Mill using a 1-mm. sieve. Each sample was analyzed for riboflavin, niacin and thiamine. Two different methods were used for determining each vitamin in one sample of every variety of legume. Greater confidence is placed in the value obtained for a vitamin assay, if the results from two methods for determining the vitamin do not vary by more than 10%.

[1] The authors are indebted to Swift and Company for a grant in support of this work.

The riboflavin methods used were the fluorometric method of Hodson and Norris ('39), as modified by Scott ('43) and the microbiological procedure of Snell and Strong ('39). The extraction of the sample for the microbiological procedure was done by digestion with takadiastase and papain according to Cheldelin, Eppright, Snell and Guirard ('42). To free the aqueous extracts of fatty material, a petroleum ether extraction was carried out at a pH of 6.7, followed by autoclaving and filtration before placing the aliquots in the assay tubes. Inconsistent and high results were obtained unless the extracts were absolutely clear.

In the determination of niacin the microbiological method of Snell and Wright ('41) and the chemical procedure of Dann and Handler ('41) were used. The extracts for the microbiological method were prepared by the same procedure as that used in the riboflavin assay. The medium was modified to include 2 μg. per tube of p-aminobenzoic acid, as suggested by Isbell ('42) when using charcoal-treated casein hydrolyzate. Low blanks were obtained by washing the Labco casein with alcohol, refluxing for 20 hours with conc. HCl and treating with Darco G-60 for 2 hours. In the chemical procedure the extracts were completely colorless before the addition of cyanogen bromide.

The methods used for the determination of thiamine were the thiochrome and the yeast fermentation procedures. The thiochrome method was that proposed by the Research Corporation Committee on the Thiochrome Method, D. J. Hennessy, Chairman ('42). The fermentation method of Schultz, Atkin and Frey ('42) was used with the folowing modification.

The unknown solution was run at two different levels, along with two other tubes containing the same levels of unknown which had been treated with sodium sulfite to destroy thiamine. Two micrograms of thiamine were added to each of the sulfite blanks, in order to bring the amount of gas produced within the same range as that of the unknown. Two standard tubes containing 2 and 4 μg. of thiamine, respectively, were used. The difference between the sulfite blanks was subtracted from the difference between the two levels of unknown, thus giving the gas produced by thiamine alone in a given amount of sample. These results agreed well with the values obtained by the thiochrome method.

## RESULTS AND DISCUSSION

A comparison of the results obtained by using two methods of analysis for each of the vitamins is presented in table 1. There is excellent agreement in all cases. The average percentage differences between the

methods for riboflavin, niacin and thiamine, respectively, are 2.67, 2.97 and 4.48. No definite positive or negative deviation in any of these sets of data was observed.

In view of these comparative data, it was thought justifiable to use only one method for the routine analysis of each vitamin. Scott's modification of the Hodson and Norris fluorometric method for determining riboflavin was chosen because it requires less time than the microbiological method, and because it eliminates the need for the removal of sub-

TABLE 1

*Comparison of results obtained by two methods of analysis for riboflavin, niacin and thiamine.*[1]

| SAMPLE | RIBOFLAVIN | | | NIACIN | | | THIAMINE | | |
|---|---|---|---|---|---|---|---|---|---|
| | Fluorometric | Microbiological | Difference | Microbiological | Chemical | Difference | Thiochrome | Yeast fermentation | Difference |
| | μg./gm. | μg./gm. | % | μg./gm. | μg./gm. | % | μg./gm. | μg./gm. | % |
| Red kidney beans | 2.16 | 2.08 | 3.70 | 25.8 | 25.8 | 0.00 | 5.90 | 6.24 | 5.45 |
| White marrow beans | 1.58 | 1.62 | 2.47 | 25.9 | 26.2 | 1.15 | 5.42 | 5.85 | 7.35 |
| Pea beans | | | | | | | | | |
| Robust | 1.89 | 1.86 | 1.59 | 29.9 | 30.4 | 1.65 | 4.78 | 4.37 | 8.58 |
| Michelite | 1.74 | 1.67 | 4.02 | 27.1 | 27.1 | 0.00 | 6.34 | 6.35 | 0.16 |
| Great Northern beans | 1.90 | 1.88 | 1.05 | 18.2 | 19.2 | 5.21 | 7.85 | 8.78 | 10.59 |
| Yelloweye beans | 1.78 | 1.72 | 3.37 | 24.8 | 23.8 | 4.03 | 9.00 | 9.25 | 2.70 |
| Pinto beans | 2.16 | 2.20 | 1.82 | 17.7 | 18.0 | 1.67 | 7.16 | 6.80 | 5.03 |
| Lima beans | | | | | | | | | |
| Baby | 1.75 | 1.76 | 0.57 | 26.3 | 27.8 | 5.40 | 6.52 | 6.80 | 4.12 |
| Fordhook | 1.70 | 1.65 | 2.94 | 23.0 | 22.4 | 2.61 | 3.66 | 3.92 | 6.63 |
| Large flat | 1.70 | 1.72 | 1.16 | 22.1 | 23.7 | 6.75 | 4.00 | 4.34 | 7.83 |
| Blackeye peas | 2.08 | 2.12 | 1.89 | 28.8 | 28.1 | 2.43 | 5.56 | 5.85 | 4.96 |
| Soybeans | | | | | | | | | |
| Chief | 3.02 | 2.96 | 1.99 | 25.8 | 26.4 | 2.27 | 10.62 | 10.45 | 1.60 |
| Richland | 3.58 | 3.39 | 5.31 | 34.8 | 35.7 | 2.52 | 12.53 | 13.66 | 8.27 |
| Manchu | 3.92 | 3.95 | 0.76 | 37.0 | 36.4 | 1.62 | 9.95 | 10.00 | 0.50 |
| Illini | 3.15 | 3.09 | 1.90 | 23.4 | 24.4 | 4.10 | 11.10 | 11.00 | 0.90 |
| Bansei | 3.50 | 3.68 | 4.89 | 29.8 | 30.3 | 1.65 | 7.64 | 7.85 | 2.68 |
| Split peas | | | | | | | | | |
| Yellow | 1.59 | 1.70 | 6.47 | 28.4 | 28.9 | 1.73 | 6.86 | 7.47 | 8.17 |
| Green | 1.74 | 1.75 | 0.57 | 36.2 | 37.1 | 2.43 | 8.01 | 8.15 | 1.72 |
| White kidney beans | 1.93 | 1.85 | 4.15 | 31.2 | 30.2 | 3.21 | 6.34 | 6.32 | 0.32 |
| Cranberry beans | 2.58 | 2.60 | 0.77 | 24.4 | 25.2 | 3.17 | 5.61 | 5.47 | 2.50 |
| Pink beans | 2.43 | 2.36 | 2.88 | 19.8 | 19.8 | 0.00 | 8.10 | 7.78 | 3.95 |
| Black turtle soup beans | 2.53 | 2.65 | 4.53 | 21.5 | 23.3 | 7.73 | 5.78 | 5.45 | 5.71 |
| Red Mexican beans | 2.25 | 2.34 | 3.85 | 18.2 | 18.5 | 1.62 | 8.12 | 7.43 | 8.50 |
| Peanuts | | | | | | | | | |
| Runner | 1.55 | 1.57 | 1.27 | 151.0 | 144.0 | 4.64 | 9.80 | 9.40 | 4.08 |
| White Spanish | 1.46 | 1.52 | 3.95 | 150.0 | 158.0 | 5.06 | 8.47 | 8.52 | 0.59 |
| Lentils | 2.56 | 2.60 | 1.54 | 25.6 | 26.8 | 4.48 | 6.93 | 6 69 | 3.46 |
| | Average | | *2.67* | Average | | *2.97* | Average | | *4.48* |

[1] Values are given for one sample of each variety of legume.

stances which interfere with the microbiological assay. In the course of the preliminary work on niacin, it was difficult to obtain agreement between the two methods when determining the niacin content of Great Northern and lima beans. There was less variation among replicates determined by the microbiological method than those obtained by the chemical procedure. Therefore, the microbiological method was selected for the routine analysis for niacin. The thiochrome method was decided upon for thiamine because more samples could be analyzed in a given period of time than by the fermentation method, since the fermentometer available for this study was a small model.

TABLE 2

*Riboflavin, niacin and thiamine content of dried legumes, as obtained.*

| PRODUCT | NO OF SAMPLES [1] | AVERAGE MOISTURE | RIBOFLAVIN | | NIACIN | | THIAMINE | |
|---|---|---|---|---|---|---|---|---|
| | | | Range | Average | Range | Average | Range | Average |
| | | % | μg./gm. | μg./gm. | μg./gm. | μg./gm. | μg./gm. | μg./gm. |
| Red kidney beans | 12 | 8.88 | 1.86–2.38 | 2.15 | 21.1–30.6 | 25.9 | 4.18– 8.60 | 6.37 |
| White marrow beans | 6 | 9.44 | 1.55–1.87 | 1.66 | 23.4–30.3 | 26.1 | 5.02– 8.48 | 6.08 |
| Pea beans | | | | | | | | |
| Robust | 5 | 5.54 | 1.74–1.89 | 1.79 | 26.5–29.9 | 28.7 | 4.78– 8.46 | 6.30 |
| Michelite | 4 | 6.90 | 1.66–1.74 | 1.70 | 26.4–34.4 | 29.1 | 6.02– 7.70 | 6.56 |
| Great Northern beans | 9 | 6.50 | 1.84–2.08 | 1.95 | 16.6–21.9 | 19.7 | 7.00– 9.60 | 8.24 |
| Yelloweye beans | 5 | 6.99 | 1.71–1.89 | 1.80 | 21.4–26.7 | 24.8 | 6.11– 9.00 | 7.40 |
| Pinto beans | 5 | 7.06 | 2.16–2.39 | 2.23 | 17.7–27.5 | 21.8 | 6.42– 7.65 | 7.18 |
| Lima beans | | | | | | | | |
| Baby | 8 | 7.74 | 1.47–2.11 | 1.74 | 20.5–26.3 | 22.7 | 3.66– 6.52 | 4.86 |
| Fordhook | 4 | 9.50 | 1.59–1.85 | 1.71 | 20.5–23.0 | 21.7 | 3.00– 3.93 | 3.57 |
| Large flat | 4 | 9.77 | 1.70–1.74 | 1.72 | 19.7–22.1 | 21.1 | 4.00– 4.78 | 4.35 |
| Blackeye peas | 5 | 6.53 | 2.05–3.04 | 2.28 | 24.6–29.5 | 27.6 | 5.52– 6.80 | 5.92 |
| Soybeans | | | | | | | | |
| Chief | 5 | 4.08 | 2.98–3.61 | 3.29 | 25.8–35.6 | 29.3 | 7.06–11.20 | 9.52 |
| Richland | 6 | 4.62 | 3.02–3.58 | 3.24 | 24.4–34.8 | 28.2 | 7.05–13.80 | 10.36 |
| Manchu | 5 | 5.30 | 2.98–3.92 | 3.32 | 19.3–37.0 | 24.6 | 6.24– 9.95 | 8.03 |
| Illini | 4 | 3.64 | 3.11–3.59 | 3.24 | 23.4–28.0 | 25.3 | 9.17–11.10 | 9.88 |
| Bansei | 5 | 6.11 | 3.01–4.12 | 3.45 | 26.0–35.3 | 29.2 | 7.41– 9.88 | 8.25 |
| Split peas | | | | | | | | |
| Yellow | 5 | 8.62 | 1.52–1.67 | 1.60 | 28.4–33.9 | 31.5 | 6.37– 7.11 | 6.92 |
| Green | 5 | 8.25 | 1.64–1.78 | 1.72 | 33.3–36.9 | 35.8 | 6.62– 8.34 | 7.56 |
| White kidney beans | 2 | 6.54 | 1.93–1.98 | 1.96 | 31.2–33.7 | 32.5 | 6.34– 6.84 | 6.59 |
| Cranberry beans | 2 | 6.24 | 2.39–2.58 | 2.49 | 24.4–24.8 | 24.6 | 5.51– 5.61 | 5.56 |
| Pink beans | 3 | 5.56 | 2.39–2.44 | 2.42 | 19.3–20.1 | 19.7 | 8.10– 9.05 | 8.68 |
| Black turtle soup beans | 2 | 7.20 | 2.53–2.54 | 2.54 | 20.7–21.5 | 21.1 | 5.70– 5.78 | 5.74 |
| Red Mexican beans | 2 | 6.73 | 2.25–2.34 | 2.30 | 17.9–18.2 | 18.1 | 8.12– 8.40 | 8.26 |
| Peanuts | | | | | | | | |
| Runner | 5 | 2.63 | 1.55–1.92 | 1.65 | 151.0–167.0 | 157.4 | 7.18–10.97 | 9.05 |
| White Spanish | 3 | 2.93 | 1.46–1.73 | 1.64 | 128.0–158.0 | 136.3 | 7.32– 8.47 | 7.86 |
| Lentils | 3 | 9.47 | 2.56–2.64 | 2.60 | 22.9–25.6 | 24.6 | 6.15– 7.67 | 6.92 |

[1] Each sample was obtained from a different source.

The results of the study of the riboflavin, niacin and thiamine content of leguminous seeds conducted by these methods are presented in table 2. In general, the values agree well with much of the data appearing in the literature. The average riboflavin content of legumes is rather consistent, ranging from 1.6 μg./gm. for yellow split peas and peanuts to 3.3 for soybeans. In the case of niacin there is little variation except for peanuts. Most legumes average between 18.1 μg./gm. for Red Mexican beans and 35.8 for green split peas. Peanuts are an outstanding exception, being one of the most potent natural sources of this vitamin, averaging 136 and 157 μg./gm. for white Spanish and runner peanuts, respectively. The average thiamine content of legumes falls within the range of 3.6 and 10.4 μg./gm. Lima beans of the Fordhook variety contain the least and soybeans the most.

No striking varietal differences are found, certainly not among the varieties of pea beans and lima beans. The greatest variation occurs in the niacin content of soybeans, split peas and peanuts.

From the results of this study it can be concluded that legumes are an excellent source of thiamine, and a good source of niacin and riboflavin. With a few exceptions, the greatest amount of all three vitamins is found in the soybean, thus substantiating its high status in the list of foods.

## SUMMARY

A study has been made of the riboflavin, niacin and thiamine content of dried leguminous seeds. Seventeen varieties of legumes were analyzed. Two different methods were used for determining each vitamin in one sample of every variety of legume. Excellent agreement was found in all cases. The routine methods used were the fluorometric method for riboflavin, the microbiological method for niacin and the thiochrome method for thiamine.

## ACKNOWLEDGMENTS

The authors wish to express their appreciation to Dr. E. V. Hardenburg, Professor of Vegetable Crops and Dr. W. H. Burkholder, Professor of Plant Pathology, Cornell University, and to the many individuals and companies who so generously contributed samples. We are also indebted to the Poultry Department of Cornell University for the use of their facilities in carrying out this investigation.

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# NUTRITIONAL STUDIES WITH THE DUCK

## I. PURIFIED RATIONS FOR THE DUCK [1, 2]

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THREE FIGURES

(Received for publication March 24, 1945)

Developments in experimental nutrition during the past decade have shown that the nutritional requirements of one species cannot be predicted from data available on other species and that the symptoms of a deficiency of the same nutrient in various animals may be markedly different. It is of interest, and perhaps unexpected, that the requirements of the chick for biotin (Hegsted et al., '42), eluate factor (Hogan and Parrot, '40; Hutchings et al., '41), and nicotinic acid (Briggs et al., '43), as well as the other members of the B-complex, appear to be quite similar to those of the monkey (Day, '44; Waisman and Elvehjem, '43), at least more similar than are the requirements of the rat and dog for these nutrients. Nutritional studies on many species are highly desirable, not only from the comparative standpoint, but also because they may find application in unexpected fields.

Only two avian species have received extensive study. At the present time, the nutritional requirements of the chick are fairly well defined. Studies on the turkey are not so complete, but the requirements for some of the vitamins and minerals and for protein can be given (Committee on Animal Nutrition, National Research Council, '44). Although the duck has been widely used as a host for experimental malarial infections, a search of the literature reveals only a few scattered references to the nutrition of this species. Pappenheimer and Goettsch ('34) have shown that vitamin E deficiency in the duck results in muscular dystrophy. Biotin deficiency and pantothenic acid deficiency have also been produced in the duck (Trager, '43) incidental to another study. The biotin

[1] The work described in this paper was done under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and Harvard University.

[2] Supported in part by a grant-in-aid from the John and Mary R. Markle Foundation.

deficiency was produced by the inclusion of raw egg white in the ration while the pantothenic acid deficiency was produced by feeding a heated ration. Although a heated ration of the type used is primarily low in pantothenic acid, it has been shown that for the chick it also contains insufficient amounts of several other factors (Waisman et al., '42).

As far as we are aware, no studies have been made with the duck using purified rations. We were interested in this species for studies on the relation of nutrition to malarial infection in a susceptible species and from the standpoint of a study in comparative nutrition. The studies reported in this paper define fairly well nutritional requirements of the duck and show that in general they are qualitatively similar to those of the chick.

## EXPERIMENTAL[1]

Day-old white Pekin ducklings were used in all of these studies. Immediately after receipt from the hatchery, they were placed in heated cages equipped with raised-screen bottoms which were 22 inches square. From two to four ducks per cage have been used, but groups of two are preferable if growth approaches optimum. Food and water were allowed ad libitum. In most of the studies reported here, the birds received no food other than the experimental ration which was supplied as soon as the ducks arrived. In more recent work, however, they have usually been fed commercial duck pellets or chick mash mixed with water for the first 2 or 3 days. This has reduced the high mortality that occasionally occurred in the first few days.

In the first experiment the birds were fed a purified diet containing 3% liver extract (D1, table 1). This diet has been used with chicks and gives good growth in this species. The effect of additional liver extract and yeast was also tested. With groups of four ducks, the average daily gain per duck during the first 10 days was as follows: Diet D1 — 14.8 gm.; Diet D1 plus an additional 3% liver extract and 5% brewer's yeast — 17.1 gm.; and commercial chick mash — 16.0 gm. The ducks were then used for studies not pertinent to this paper, but it was apparent that good growth, although perhaps not optimum, could be obtained with Diet D1.

Another approach was made in experiment 2 by using a highly purified diet (D2, table 1) as the basal ration. This ration is fairly adequate for the rat but completely unsatisfactory for the chick. The results obtained with this diet and supplements of 10% gelatin, 3% liver extract,

[1] The synthetic vitamins were supplied by Merck and Company, Rahway, New Jersey, and the liver extract was supplied by The Wilson Laboratories, Chicago, Illinois.

0.5% arginine hydrochloride, and 20 μg. biotin per 100 gm. in various combinations are shown in figure 1. As for the chick, Diet D2 is completely inadequate for growth in the duckling. Biotin, liver extract, and gelatin all appear to be beneficial. Apparently arginine at 0.5% does not completely replace gelatin. In further studies in which the effect of additional casein was compared with gelatin, Diet D2 was supplemented with liver extract and biotin (Diet D4). The average weight in grams after 20 days on this diet (D4) and with supplements of casein and gelatin was as follows: D4 — 390; D4 plus 10% casein — 602; D4 plus 10% gelatin — 666. It thus appears that gelatin is superior to

TABLE 1

*Composition of diets.*

| CONSTITUENT | D1 | D2 | D3 | D4 |
|---|---|---|---|---|
| | % | % | % | % |
| Sucrose | 55 | 70 | 60 | 66 |
| Casein, SMA, "vitamin-free" | 20 | 18 | 18 | 18 |
| Salts IV [1] | 5 | 5 | 5 | 5 |
| Corn oil | 5 | 4 | 4 | 4 |
| CaHPO4 | 1 | 1 | 1 | 1 |
| Cod liver oil | 1 | 2 | 2 | 2 |
| Gelatin | 7 | | 10 | |
| Calcium gluconate | 2 | | | |
| Cellu flour | 1 | | | |
| Liver extract [2] | 3 | | | 4 |
| Choline chloride | 0.3 | 0.3 | 0.3 | 0.3 |
| Vitamin mixture [3] | + | + | + | + |
| Biotin, 15 μg. per 100 gm. | + | | | + |

[1] Hegsted, D. Mark, R. C. Mills, C. A. Elvehjem and E. B. Hart 1941 Choline in the nutrition of chicks. J. Biol. Chem., vol. 138, p. 459.

[2] Wilson's Fraction "L."

[3] A mixture supplying 400 μg. thiamine hydrochloride, 400 μg. pyridoxine hydrochloride, 800 μg. riboflavin, 1.5 mg. pantothenic acid, 3.0 mg. nicotinic acid, and 100 μg. 2-methyl-1, 4-napthoquinone and 10 mg. α tocopherol per 100 gm. of ration.

casein in supplementing the ration, and certainly amino acids in addition to those supplied by 18% casein are required. If the amino acid composition is satisfactory, however, 20% protein is sufficient since the duck pellets which give excellent growth contain only 20.3% crude protein.

The need for biotin and a factor in liver extract is more clearly shown in figure 2. The basal diet used in this experiment was the highly purified Diet D2 plus 10% gelatin and is given as Diet D3 in table 1. The liver extract used in this experiment was extracted three times with boiling absolute alcohol to remove biotin. Practically no growth was

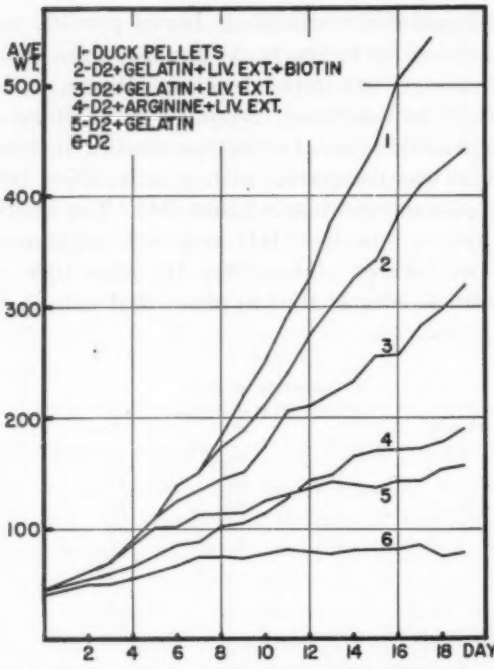


Fig. 1 The growth curves of ducks on the purified diet (D2) only and with various supplements compared to duck pellets. The supplements supplied 10% gelatin, 3% liver extract (Wilson's fraction "L"), 0.5% arginine hydrochloride, and 20 μg. biotin per 100 gm.

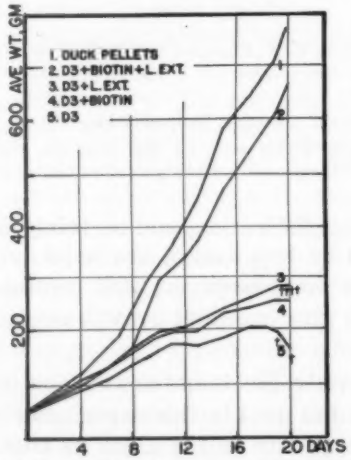


Fig. 2 Growth curves of ducks on Diet D3 plus 3% liver extract and 20 μg. biotin per 100 gm. and these supplements together. The liver extract was previously extracted with absolute alcohol to remove biotin.

obtained with supplements of either biotin or liver extract alone, but the combination gave good growth. As in the previous experiment, the growth is still somewhat less than is obtained with a good commercial ration. The deficiency symptoms in the groups lacking biotin and liver factor were not remarkable other than the very poor growth. No signs of dermatitis were seen in the biotin deficient birds. Those lacking the liver factor appeared to develop a slight spastic paralysis after the first few days; however they improved spontaneously.

TABLE 2

*The effect of various supplements on food consumption and utilization.*

| DIET [1] | TIME | GAIN IN WEIGHT | FOOD INTAKE PER GM. GAIN | GAIN PER GM. FOOD |
|---|---|---|---|---|
| | *days* | *gm./day* | *gm.* | *gm.* |
| Diet II | 9 [2] | 2.1 | 8.5 | 0.12 |
| Diet II + 10 gm. gelatin | 9 | 4.7 | 7.9 | 0.13 |
| Diet II + 0.5 gm. arginine + 3 gm. liver extract | 9 | 10.0 | 3.0 | 0.33 |
| Diet II + 10 gm. gelatin + 3 gm. liver extract | 9 | 13.8 | 2.8 | 0.35 |
| Diet II + 10 gm. gelatin + 3 gm. liver extract + 40 μg. biotin | 9 | 24.9 | 2.35 | 0.43 |
| Duck pellets | 9 | 33.4 | 1.80 | 0.55 |
| Diet III | 15 [3] | 7.8 | 6.9 | 0.15 |
| Diet III + 40 μg. biotin | 15 | 9.5 | 4.0 | 0.25 |
| Diet III + 3 gm. liver extract | 15 | 12.1 | 3.36 | 0.30 |
| Diet III + 40 μg. biotin + 3 gm. liver extract | 15 | 33.5 | 2.34 | 0.43 |
| Chick mash | 15 | 35.2 | 2.23 | 0.45 |
| Duck pellets | 15 | 41.0 | 2.05 | 0.49 |

[1] Supplements given as additions per 100 gm. of ration.

[2] Experiment 2. From fifth to fourteenth day.

[3] Experiment 3. From second to seventeenth day.

The effect of the various supplements in the experiments with Diets D2 and D3 is clearly shown in table 2 in the data on food consumption. On the basal diets the food was only 12 to 15% efficient (gain in weight per gram of food), while the duck pellets were 50% efficient. As was found by weight measurements, the purified ration approaches but does not equal the results obtained on the commercial rations.

The factors required by the duck which are present in liver extract are probably the same as those required by the chick. It has been shown that they are present in an eluate prepared by the treatment of the liver extract with fuller's earth at pH 3 and elution with dilute ammonia. A concentrate of "L. casei factor" [4] was tried curatively as shown in

[4] Kindly supplied by Dr. E. L. R. Stokstad, Lederle Laboratories, Pearl River, New York. This preparation contained 0.85 mg. of L. casei factor in 17 mg. of solids according to Dr. Stokstad.

figure 3. The basal diet in this experiment was D3 plus 40 μg. of biotin per 100 gm. of ration, and the curve represents the average weight of three ducks in the group. It is apparent that the oral supplements of this concentrate resulted in a marked growth response; larger amounts at the beginning of supplementation might have made this more marked. Ducks deficient in the eluate factor show a severe anemia although this is much less consistent than is the growth failure. Hemoglobin values of 4 and 5 gm. per cent and hematocrit reading below 10% are observed in severe deficiency.

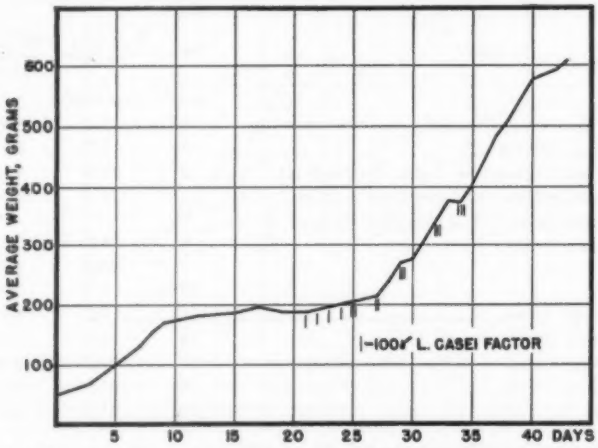


Fig. 3 The effect of supplements of L. casei factor on ration D3 plus biotin.

## DISCUSSION

The studies reported in this paper indicate that the nutritional requirements of the duck are qualitatively similar to those of the chick. The duck has a very rapid rate of growth which appears to make it very susceptible to nutritional deficiencies. In studies on specific deficiencies to be reported later, it has been found that deficiencies of most of the vitamins so far studied may be easily produced by the omission of the appropriate vitamin from Diet D3 plus liver extract and biotin. In practically all instances the deficiency is clearly evident within a week after the experiment is started.

The very high efficiency of food utilization noted in these studies has been found previously. Titus ('39) gives the pounds of feed required to produce a live weight of one pound in various birds as follows: White Leghorns, 3.18; Rhode Island Red chickens, 2.53; turkeys, 2.20; and

White Pekin ducks, 2.01. It will be of interest to determine whether or not this high efficiency is associated with unusual (high or low) vitamin requirements.

Certain disadvantages of the duck as an experimental animal are also apparent. Considerable variation within a group is noted and is at least as great if not greater than is found with chicks. This together with the larger amounts of purified ration consumed, greater space requirements as compared with the chick, and the fact that it is a messy animal to work with should be recognized.

Good growth on purified rations may be obtained with the duck, but as with the rat and the chick, it falls somewhat short of that resulting from the feeding of good commercial rations. With regard to the factors in liver extract required by the duck, it is apparent that they are similar if not identical to those required by the chick. Pfeffner et al. ('43) found that vitamin $B_c$ was the only critical material supplied by such preparations on their purified chick diet. However, Briggs et al. ('44) state that the eluate factors are multiple, one factor being required primarily for growth and another for feather development, and suggest that both of these factors may be distinct from vitamin $B_c$. The ducks in these studies which were fed the purified rations definitely did not show as good feather development as those fed the crude duck pellets. However, this observation is complicated by the fact that the purified rations are composed largely of sucrose and are sticky when wet. The ducks always succeed in getting considerable of this sticky ration on them, especially around the head and neck. This may be somewhat irritating and in any event makes a classification of feather development difficult. Some feather pulling in these animals has also been noted.

It has been shown previously that the high arginine and glycine requirement of the chick is due chiefly to the amino acids required for feather formation (Hegsted et al., '41). Although a requirement of the duck for glycine has not been shown, the requirement for additional arginine, above that supplied by 18% casein, is undoubtedly due to the rapid rate of growth since feather formation is very slight during the first few weeks of growth. From a comparative standpoint, it is also interesting that the birds deficient in biotin show no external symptoms comparable to the dermatitis found in the biotin deficient chick (Hegsted et al., '40).

## SUMMARY

1. The duck is an extremely rapidly growing animal and on an adequate ration gains approximately 40 gm. a day during the first 3 weeks of life with an efficiency of food utilization of 50%.

2. Good growth, but somewhat less than optimum, occurs on purified diets containing only liver extract as the crude material. The factor in liver extract is similar or identical to the eluate factor or factors required by the chick. As in the chick, a deficiency results in an anemia.

3. The duck is similar to the chick in that additional amino acids above those supplied by 18% casein are required for good growth. Gelatin is superior to casein as a source of these amino acids.

4. Biotin is required by the duck. No symptoms other than very poor growth were noted in this deficiency.

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# STUDIES ON THE REQUIREMENTS OF THE MONKEY FOR RIBOFLAVIN AND A NEW FACTOR FOUND IN LIVER [1]

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THREE FIGURES

(Received for publication April 2, 1945)

Recent reports from this laboratory have demonstrated the value of the monkey as an experimental animal in nutritional research. Waisman, Rasmussen, Elvehjem and Clark ('43) described a purified diet which is capable of maintaining monkeys in excellent health for extended periods of time, and the use of this diet has made it possible to study uncomplicated vitamin deficiencies. Studies on "folic acid" (Waisman and Elvehjem, '43), biotin (Waisman, McCall and Elvehjem, '45) and thiamine (Waisman and McCall, '44) deficiencies have already been reported. A preliminary report has been made on riboflavin studies with the monkey (Waisman, '44) and in this paper we wish to report further observations on this deficiency.

## EXPERIMENTAL

The housing, care and handling of the monkeys have been described previously (Waisman et al., '43). The basal diet (M-2) consisting (in %) of sucrose 73, Labco casein 18, salts IV 4, cod liver oil 3 and corn oil 2, was fed ad libitum. The following vitamin supplement was given daily: thiamine hydrochloride 1 mg., pyridoxine hydrochloride 1 mg., calcium pantothenate 3 mg., nicotinic acid 5 mg., choline chloride 25 mg., para-aminobenzoic acid 50 mg., i-inositol 50 mg., biotin 20 μg., and ascorbic acid 25 mg. In addition a norite eluate concentrate prepared according to Hutchings et al. ('41) was fed equivalent to 5 gm. of "solubilized liver residue" per animal per day.

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by grants from the National Foundation for Infantile Paralysis, Inc., New York, and the Commercial Solvents Corp., Terre Haute, Indiana.

We are indebted to The Wilson Laboratories, Chicago, Illinois, for the various liver products and to Merck and Co., Inc., Rahway, New Jersey, for the synthetic vitamins.

The basal diet was analyzed for riboflavin by both the microbiological method of Snell and Strong ('39) as modified by Strong and Carpenter ('42) and the fluorometric method (Andrews, '43) and found to contain 0.3 μg. per gram. Since the results of both these methods checked very well, the fluorometric method was used in subsequent analyses because it was more convenient. The norite eluate concentrate contained between 1 and 2 μg. of riboflavin per gram equivalent of the original solubilized liver residue. The maximum amount of riboflavin which each animal received from this source each day was therefore 10 μg.

As previously demonstrated (Waisman, '44) the monkeys developed a "freckled type" of dermatitis in 6 to 8 weeks after being placed on the basal diet. Soon thereafter large areas of the body became covered with dark scabs which promptly regressed when riboflavin was administered. Among the other noticeable changes in the more severe cases were incoordination in the use of the limbs, faulty grasping reflex, impaired vision and a scanty hair coat. In two cases graying of the hair was observed.

Hemoglobin determinations, red blood cell and white blood cell counts were made on blood drawn from the marginal vein of the ear. Hemoglobin was determined in the Evelyn photoelectric colorimeter and red and white cell counts were made in the usual manner.

The hemoglobin level of the blood and the red cell count showed a decrease about a week after the onset of the dermatitis, and the white blood cell counts decreased to a lesser extent. The hemoglobin and red blood cell counts reached the lowest level about 5 weeks later. When riboflavin was not administered the monkeys eventually died.

In table 1 are shown the blood values for some of the monkeys before the onset of the deficiency, the lowest values at the height of the deficiency, and the levels reached after 6 weeks of riboflavin therapy. It is evident that a definite anemia appeared as part of the syndrome of riboflavin deficiency. The hemoglobin and red blood cells decreased in approximately the same ratio indicating a hypochromic, normocytic type of anemia. The white cell counts responded to riboflavin therapy to a lesser extent than did the hemoglobin and red cell counts.

These changes are clearly indicated in figure 1 which shows the blood picture of a typical monkey. It is interesting to note that the decrease in weight is not as sharp as in "folic acid" deficient monkeys (Waisman and Elvehjem, '43). This may be partly explained by the fact that the monkeys never developed anorexia during the course of the deficiency. There is, however, a definite weight response when riboflavin is administered to the deficient animals.

One hundred micrograms per day of riboflavin was found to be the minimal amount which brought about the regression of the symptoms in all the monkeys. Fifty micrograms had variable effects, being effective in the milder cases but ineffective in more advanced cases. One hundred micrograms generally brought about the remission of the dermatitis in 10–14 days, although in some cases only 5 days elapsed before remission occurred.

In several cases it required a week of riboflavin therapy before an increase in hemoglobin and red cells was apparent but after the initial lag the increase was rapid.

TABLE 1

*Values for the blood constituents of monkeys before, during and after riboflavin deficiency.*

| MONKEY NO. | HEMOGLOBIN | | | RED BLOOD CELLS | | | WHITE BLOOD CELLS | | |
|---|---|---|---|---|---|---|---|---|---|
| | Before deficiency | During deficiency | After therapy | Before deficiency | During deficiency | After therapy | Before deficiency | During deficiency | After therapy |
| 53 | 13.4 | 4.9 | 10.73 | 5.5 | 2.0 | 3.10 | 11.0 | 8.9 | 10.6 |
| 81 | 13.0 | 7.72 | 12.76 | 6.1 | 3.02 | 3.25 | 11.0 | 6.09 | 9.8 |
| 82 | 12.9 | 8.0 | 12.41 | 6.09 | 1.7 | 3.80 | 13.8 | 11.8 | 10.0 |
| 125 | 13.11 | 9.22 | 12.06 | 4.30 | 2.0 | 3.30 | 14.6 | 8.0 | 12.6 |
| 6 | 13.38 | 8.63 | 12.14 | 4.28 | 2.7 | 3.20 | 14.6 | 6.2 | 12.8 |
| 13 | 13.89 | 5.07 | 11.37 | 4.96 | 1.89 | 3.70 | 15.6 | 11.0 | 12.6 |
| 128 | 13.50 | 8.99 | 12.06 | 4.52 | 3.60 | 3.20 | 14.7 | 12.4 | 9.6 |
| 135 | 13.50 | 5.54 | 12.14 | 4.10 | 1.90 | 3.16 | 11.4 | 4.6 | 10.4 |
| 166 | 13.89 | 6.76 | 12.76 | 3.95 | 2.0 | 3.25 | 14.2 | 9.2 | 10.0 |
| 164 | 14.28 | 7.72 | 11.48 | 4.30 | 2.2 | 3.30 | 10.0 | 6.8 | 9.2 |

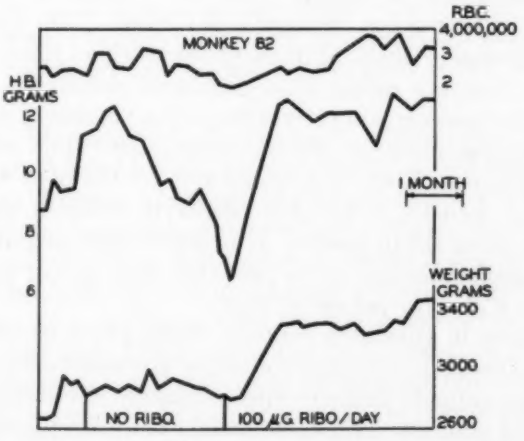


Fig. 1 Effect of riboflavin deficiency on weight, hemoglobin and red blood cell level of typical monkey, and the response to riboflavin therapy.

The deficient monkeys were examined with a slit lamp for corneal vascularization, and in none of them were any signs of corneal vascularization or opacity evident. In fact some of the control monkeys on a complete ration showed more vascularization. In no case did any cheilosis or tongue involvement appear.

### *Liver studies*

Three of the deficient monkeys were sacrificed and upon autopsy no gross pathology except yellow livers was evident. The livers were removed, dried, and analyzed for fat. At the same time, livers from two monkeys which had died of acute "folic acid" deficiency were analyzed for fat for comparison. The riboflavin content of the various livers was also determined, and all of the data are presented in table 2.

TABLE 2

*The fat and riboflavin content of the livers of riboflavin deficient and non-riboflavin deficient monkeys.*

| | RIBOFLAVIN DEFICIENT | | | NON-RIBOFLAVIN DEFICIENT | |
|---|---|---|---|---|---|
| MONKEY NO. | 57 | 80 | 64 | 32 | 63 |
| Fat (% dry basis) | 26.22 | 47.01 | 42.5 | 23.96 | 12.75 |
| Riboflavin (mg./100 gm. dry basis) | 3.62 | 3.27 | 2.02 | 8.00 | 9.00 |

It will be noted that a distinct fatty liver condition existed in the riboflavin deficient monkeys. Sebrell and Onstott ('38) also noted yellow livers in riboflavin deficient dogs. Other workers have claimed that this is due to inanition rather than riboflavin deficiency. The results in the case of the monkey are interesting since riboflavin deficient monkeys do not develop anorexia. On the other hand "folic acid" deficient monkeys do show a reduced food intake and yet the liver fat values are distinctly lower than the values for riboflavin deficient monkeys. The reason that the liver fat in monkey 57 is not as high as in the other two monkeys is undoubtedly due to the fact that this animal was on the deficient diet for a shorter period of time.

The differences in riboflavin content of the livers of riboflavin deficient and non-riboflavin deficient monkeys are noteworthy. The value for the deficient animals drops to approximately one-third of the value for non-deficient animals. This corresponds well with the work of Kuhn, Kaltschmitt and Wagner-Jauregg ('35) and Vivanco ('35) in which they showed that the body conserves its store of riboflavin, and that

even in rats that die for lack of this vitamin, the amount in the liver, kidney and heart is still about one-third of the normal level.

*Excretion studies*

Four of the monkeys were placed in metabolism cages which were similar to the regular cages except that they are equipped with a funnel-shaped, metal bottom for the collection of urine. A fine screen was placed below the regular floor of the cage in order to collect the feces. Sheets of tin were placed on the screen floors of the metabolism cages under the food cups to prevent food from falling into the urine. Whenever the monkeys showed diarrhea the urine samples were disregarded. The urines were stored in brown bottles with measured amounts of glacial acetic acid and toluene added and placed in the cold room until they were assayed. The feces were collected and dried at 45°C. for 24 hours in a vacuum oven. After drying the feces were placed in brown bottles and stored in the cold room until they were assayed. The fluorometric method of assay was employed.

The riboflavin content of the feces was expressed as micrograms per dry gram rather than total weight of feces since it was not possible to remove quantitatively all the feces from the screen floor of the cage. However, the values obtained remained constant regardless of the intake or state of deficiency of the animals, the average value being 30 μg. of riboflavin per gram of dry feces. Since the daily total excretion via the feces exceeded in most cases the daily intake of riboflavin, it seems apparent that the greater part of the riboflavin in the feces is dependent upon the synthesis of this vitamin by the intestinal flora. Apparently most of the riboflavin synthesized by the flora is not available to the monkey.

*Monkey 53.* This animal had been on the deficient diet until dermatitis and the abnormal blood picture appeared; 100 μg. of riboflavin were then given daily for 20 days. At the end of this period, the dermatitis had disappeared and the hemoglobin and red cell content of the blood had increased. The monkey was then put on a riboflavin deficient diet and the urine and feces collected during 24-hour periods. The only source of riboflavin was the basal ration from which the monkey received an average of 40 μg. per day. For 2 weeks the daily output of riboflavin in the urine was relatively high, averaging 60–90% of the intake. After the third week the average daily output was 12% of the intake and remained at this level until a single dose of riboflavin was given. At this time the excretion increased to about 50% of the intake. Shortly after the ad-

ministration of the single dose the excretion fell to 12% of the intake and symptoms of riboflavin deficiency began to appear.

*Monkey 82.* Monkey 82 was on a regimen similar to that of monkey 53. It had been on a deficient diet and after the symptoms of riboflavin deficiency appeared, it was given 500 μg. of riboflavin daily for 15 days. At the end of this period the dermatitis had cleared up and the blood picture approached normal. Riboflavin was then discontinued and excretion studies started 3 days later. For the first 5 weeks the daily output of riboflavin by way of the urine averaged 60–95% of the intake. By the seventh week the 24-hour riboflavin output averaged 15% of the intake. It remained at a level of 10–15% until a test dose of riboflavin was given. At this point the output rose to 60% of the dietary intake.

The results of these two experiments agree well with recent studies by two groups of workers. Williams et al. ('43) found that the daily output of riboflavin dropped to a level of 14% of the intake during the depletion period in human subjects.

Keys and coworkers ('44) working with young men found that during the depletion period the 24-hour output dropped to 12% of the intake. When a test dose was administered the output rose to 54% of the intake.

It is also significant to note that none of the above workers found vascularization of the cornea or cheilosis among the deficient men.

*Monkey 64.* Monkey 64 had been on the deficient diet and after the typical symptoms appeared, therapy of 50 μg. per day was started. Excretion studies were started 12 days after therapy was instituted. The average daily output varied between 40–60% of the intake for a 3-month period.

*Monkey 81.* Monkey 81 was on a regimen similar to that of monkey 64. It too was put on metabolism studies 2 days after riboflavin therapy was instituted. Previous to that it had been in the deficient state. This monkey was also receiving a daily supplement of 50 μg. of riboflavin a day in addition to what it received in the basal ration. For the entire period the daily output varied between 30–80%.

From the above two studies it would appear that the deficient animal does not retain all the ingested riboflavin.

Feder, Lewis and Alden ('44) have suggested that when the excretion of riboflavin is expressed in micrograms per milliliter of urine, a more accurate estimation of the body's supply of riboflavin is possible. They assumed that an excretion below 3 μg. per milliliter shows a state of deficiency. In our study an attempt was made to express excretions on this

basis but results were variable. A better correlation was obtained when the results were expressed as total riboflavin output for a 24-hour period. The discrepancies might be partially explained by the fact that frequently the monkeys spilled their drinking water into the urine collection jars. This would make any results based on micrograms per milliliter of urine meaningless.

A summary of the excretion studies is presented in table 3.

TABLE 3

*Urinary excretion of riboflavin.*

| WEEK | AVERAGE DAILY RIBOFLAVIN EXCRETION IN MICROGRAMS | | | | AVERAGE DAILY EXCRETION AS PER CENT OF INTAKE | | | |
|---|---|---|---|---|---|---|---|---|
| | Monkey | | | | Monkey | | | |
| | 53 | 82 | 64 | 81 | 53 | 82 | 64 | 81 |
| 1 | 36 | 38 | 52.2 | 72 | 90 | 95 | 58 | 80 |
| 2 | 33.6 | 32 | 43.3 | 43.3 | 84 | 80 | 47 | 47 |
| 3 | 28 | 36 | 36 | 55.8 | 70 | 90 | 40 | 62 |
| 4 | 6 | 26.8 | 48.6 | 67.5 | 15 | 67 | 54 | 75 |
| 5 | 4.8 | 24 | 55.8 | 45 | 12 | 60 | 62 | 50 |
| 6 | 5.6 | 6 | 45 | 36 | 14 | 15 | 50 | 50 |
| 7 | 5.6 | 7.2 | 40.5 | 33.3 | 14 | 18 | 45 | 37 |
| 8 | 4.8 | 5.6 | 43.2 | 18 | 12 | 14 | 48 | 20 |
| 9 | 4.8 | 6 | 45 | 43.3 | 12 | 15 | 50 | 47 |
| 10 | 4.8 | 5.6 | 54 | 64.8 | 12 | 14 | 60 | 72 |
| 11 | 75.6 | 351 | | | 54 | 65 | | |
| | Total daily intake of riboflavin | | | | | | | |
| | 40 [1] | 40 [2] | 90 | 90 | | | | |

[1] Increased to 140 at 11th week.

[2] Increased to 540 at 11th week.

### *Studies on a new factor for the monkey*

As has already been demonstrated, a part of the syndrome of riboflavin deficiency in monkeys is the occurrence of a hypochromic, normocytic anemia. Upon administration of riboflavin there is an increase in hemoglobin, red cell and white cell content of the blood. However, a plateau is soon reached in both the weight and blood responses. If the blood picture had reached normal values this phenomenon would be expected. However, the hemoglobin content did not reach accepted normal levels. A survey of the literature revealed conflicting data on the exact normal blood picture of the rhesus monkey. Shukers et al. ('38) reported a study upon nineteen monkeys comparing their results with previously published works and their values are as follows: hemoglobin 10.0–13.5 gm. %; red blood cells 4.6–5.8 millions per cubic millimeter;

white blood cells 9.7–20.5 thousands per cubic millimeter. Two Indian workers, Rao and Rao ('40), studying macacus sinicus reported a large range in the normal blood picture of the monkey.

Since the diets which the various workers used and the methods of determination varied greatly, it is difficult to evaluate the results. Studies in our laboratory on normal, healthy, young monkeys receiving 3% liver powder in addition to the basal ration showed a normal range as follows: hemoglobin 14–15.5 gm. %; red blood cells 4–5.5 millions per cubic millimeter; white blood cells 10–25 thousands per cubic millimeter. Referring again to table 1 we see that the plateau occurs at a level well below that which can be considered normal. This plateau occurred after treatment of every deficient monkey, whether the daily therapeutic dose was 100, 500 μg, or 1 mg. of riboflavin.

These results indicated that other factors were necessary to produce a normal growth and concentration of blood constituents. Therefore, monkey 125, after reaching this plateau, was placed on the basal ration M-2 plus 3% 1:20 liver extract, the liver powder being added at the expense of the dry part of the ration. After 3 months on this ration there was no apparent improvement.

The monkey was then placed on the M-2 basal ration for 2 weeks, after which it was changed to M-2 basal plus 3% whole liver powder. Within a week there was a perceptible increase both in weight and blood constituents. Within 3 weeks the blood hemoglobin level rose from 12 gm. to 14.5 gm. per 100 ml. and the red cell count increased proportionately. A sharp increase in weight also occurred.

Two other monkeys, numbers 53 and 128, which were at the plateau stage after recovering from a riboflavin deficiency were then placed on the M-2 basal plus 3% whole liver substance. In monkey 128 the hemoglobin increased from a level of 12.4 to 14.7 gm. %. The red cell count rose from 3.4 to 4.8 millions per cubic millimeter.

After 9 weeks on this regimen, the animal was returned to the M-2 basal without the liver. The weight continued to increase for about 3 weeks at which time it showed a plateau. The blood picture fell more rapidly than the weight and the hemoglobin value returned to 12.3 gm. % in 3 weeks.

The effect of the whole liver substance on monkey 53 is shown in figure 2. Here again a rapid and sharp increase in weight, hemoglobin and red cells content was demonstrated. It is also interesting to note the increase in the white blood cell count. This increase in the blood constituents and weights occurred in every case without exception when a monkey having reached the plateau stage was given the ration con-

taining 3% whole liver substance. In a period of 3½ months, monkey 53 gained over 2 kg. in weight. It had gained 200 gm. over a similar period when riboflavin alone was given without the liver. In 1 month's time on whole liver substance the hemoglobin increased from a level of 10.5 to 14.6 gm. %, the red cells from 3.1 to 5.1 millions per cubic millimeter, and the white cells from 11 to 16 thousands per cubic millimeter. Here again the discontinuance of the liver substance produced a plateau in both the weight and blood elements and a downward trend after a few weeks.

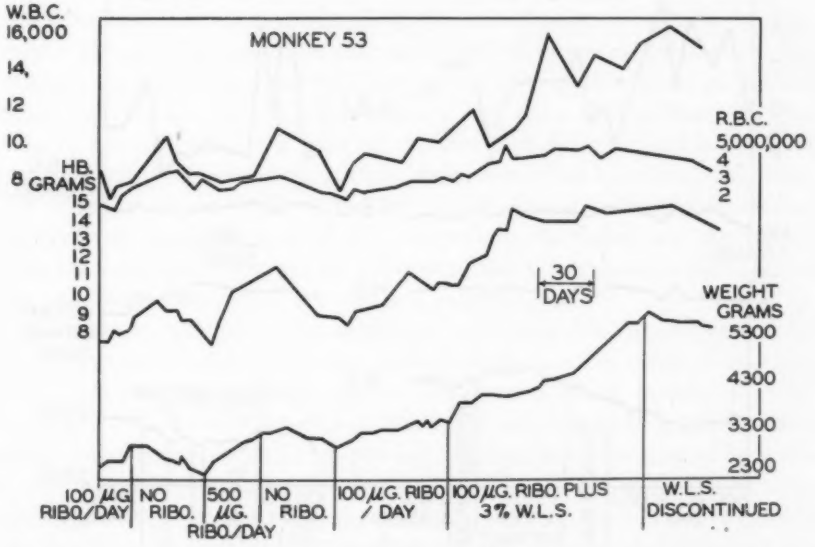


Fig. 2 Curve representing effect of whole liver powder on weight, hemoglobin, red and white cell count in typical riboflavin deficient monkey which showed a plateau following riboflavin therapy.

Scott and coworkers at Cornell ('44) reported that pyridoxine treated with hydrogen peroxide promoted growth and more particularly hemoglobin formation in chicks. Accordingly hydrogen peroxide treated pyridoxine or "pseudo-pyridoxine" was prepared by the method of Carpenter and Strong ('44) and fed to two monkeys, numbers 128 and 82, at a level equivalent to 1 mg. of the original pyridoxine per day. From figure 3 it can be seen that the preparation had no effect in monkeys 82 and 128 on growth or the blood picture over a period of 3 and 4 weeks, respectively.

Monkey 82 was then put on a regimen of the M-2 basal plus 3% 1:20 liver extract powder. From figure 3 it is apparent that this had no additional effect. Any differences may be accounted for by the fact that different batches of liver powder were used in each case.

In a report from Puerto Rico (Report of Director, '43) there appeared a note that the iron content of ordinary laboratory rations was too low for monkeys. Accordingly, two monkeys, numbers 164 and 128, were given in addition to the iron supplied by the basal ration 10 mg.

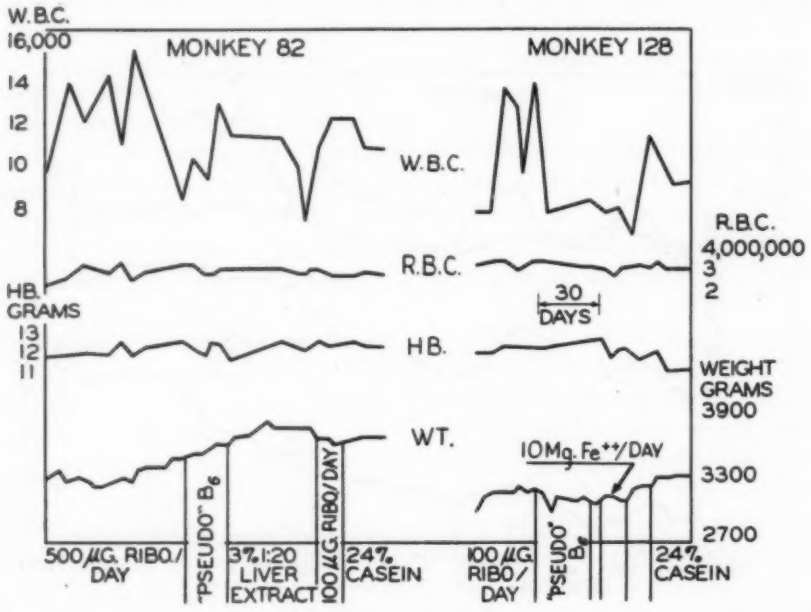


Fig. 3 Effect of various supplements on hemoglobin level, red and white blood cell count, and weight of two typical monkeys which reached the plateau stage following riboflavin therapy. Supplements added in addition to riboflavin.

of iron in the form of hydrated ferrous ammonium sulfate. From figure 3 we see that the additional iron had no hemoglobin building or growth promoting properties. A similar effect was noticed with monkey 164.

In order to determine if the protein level of the ration was adequate for optimum growth and hemoglobin production, monkeys 128 and 82 were given a ration containing 24% casein, the extra casein being added at the expense of the sucrose. It is evident from figure 3 that adding extra protein had no effect either on the blood picture or weight response.

Three monkeys, numbers 82, 128 and 164, were fed the M-2 basal plus 3% extracted liver residue, the fraction left when 1:20 liver extract is made from whole liver powder. However, after 4 weeks on this ration none of the animals responded in any way.

One monkey was maintained on a "complete" purified ration M-2 plus all the known vitamins and the norite eluate concentrate. After 18 months on this ration the monkey suddenly lost weight and was saved through the use of whole liver powder therapy.

## DISCUSSION

Classical studies have resulted in the association of riboflavin deficiency with cheilosis and corneal vascularization and slit lamp studies of the eye are routinely used to diagnose the lack of this vitamin. Recent studies (Williams et al., '43; Keys et al., '44) have shown that this syndrome is not necessarily a part of riboflavin deficiency and have indicated that many cases showing these symptoms fail to respond to riboflavin therapy. This study indicates that cheilosis or corneal involvement was not necessarily a part of the riboflavin deficiency syndrome. However, these studies must be differentiated from chronic deficiencies more generally encountered in clinical practices.

Although no attempt was made to establish the exact riboflavin requirement of monkeys, certain calculations can be made regarding the requirement when a high sugar diet is used. It is obvious that all monkeys developed a deficiency on the basal ration supplying 40 μg. per monkey per day, or about 12 μg. per kilogram of body weight. The addition of 25 μg. failed to give a response in the deficient animals, but the addition of 50 μg., or a total intake of 90 μg. per day, cured the symptoms in the monkeys with the milder symptoms and allowed considerable excretion in the urine. Thus the requirement of a young monkey is probably 25 to 30 μg. per kilogram of body weight per day. Whether the amount for maintenance of adult monkeys is less cannot be determined from the present data, but it is entirely possible that the requirement may be as low as 15 to 20 μg. per kilogram. These figures are of the same magnitude as the riboflavin requirement of dogs as established by Spector et al. ('43).

It has become evident during the course of these studies that the synthetic ration used in these studies is not complete for the monkey. A factor (or factors) which is found in whole liver powder appears to be necessary for optimum growth and blood regeneration. A riboflavin deficiency precipitates the need for this factor (or factors). The administration of riboflavin results in a weight gain accompanied by a sig-

nificant rise of hemoglobin level and red cell count but these increases do not attain accepted normal values. Such monkeys can then be used as assay animals for the factor (or factors) in whole liver powder This factor (or factors) is evidently labile since commercial extraction procedures used to prepare 1 : 20 liver extract and extracted liver residue from whole liver destroy nearly all activity. Recently Day and coworkers ('45) reported that injection of highly purified preparations of the Lactobacillus casei factor resulted in a return of the total white blood cell and granulocyte counts to normal levels in vitamin M deficient monkeys. However, there was little improvement in the hemoglobin levels and no weight data were given. That our factor described above appears to be different is evident from the fact that the norite eluate concentrate used in our ration is a rich source of the Lactobacillus casei factor.

## SUMMARY

Rhesus monkeys were placed on a riboflavin deficient diet and after 6 to 8 weeks a loss in weight became apparent and soon thereafter a freckled type of dermatitis appeared first on the face, then on the hands, legs and groin. Shortly after the appearance of the dermatitis the hemoglobin, red cells and to a lesser extent the white cell level showed a decrease, a hypochromic normocytic type of anemia developing. With the administration of riboflavin growth was resumed, the dermatitis regressed and the anemia showed some improvement.

Excretion studies showed that normal monkeys excrete riboflavin daily at the same range as normal humans and that on a deficient diet the daily output drops to 10–15% of the daily intake. Saturation tests fail when applied as a diagnostic tool to determine riboflavin deficiency in the monkey.

Fatty livers which cannot be explained on the basis of inanition were demonstrated in monkeys that had died of riboflavin deficiency.

The minimal daily requirement for young monkeys appears to be approximately 25 to 30 μg. per kilogram of body weight.

Shortly after the administration of riboflavin to deficient monkeys a plateau was reached in the values for their hemoglobin, red and white cells and weights below normal levels. Iron, "pseudopyridoxine," 1 : 20 liver powder, extracted liver residue, and increasing the casein level to 24% proved ineffective in restoring the blood picture and weight to a normal level. However, a factor (or factors) found in whole liver substance was necessary at a level of 3% of the ration for optimum growth and blood regeneration.

ACKNOWLEDGMENT

We wish to acknowledge the assistance of Arthur M. Ingebritsen for his help in the blood and excretion studies.

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